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**STUDIES ON THE
NATURAL FLUORESCENCE
OF WOOL AND WOOL GREASE**

by

Stephen Collins

**A thesis submitted for the
Degree of Doctor of Philosophy
at the**

**City University,
Department of Chemistry**

September 1992

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PSALM 23

A Psalm of David

¹THE LORD is my shepherd; I shall not want.

²He maketh me to lie down in green pastures:

he leadeth me beside the still waters.

³He restoreth my soul:

he leadeth me in the path of righteousness for his name's sake

⁴Yea, though I walk through the valley of the shadow of death, I will fear no evil:

for thou art with me;

thy rod and thy staff they comfort me.

⁵Thou preparest a table before me in the presence of mine enemies:

thou anointest my head with oil;

my cup runneth over.

⁶Surely goodness and mercy shall follow me all the days of my life:

and I will dwell in the house of the LORD for ever.'

DECLARATION

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S. Collins

September 1992

ABSTRACT

The principal aims of the work were to further the current knowledge of the natural fluorescence of wool and wool wax, to characterise the species responsible and to thoroughly investigate the light induced changes.

The natural visible fluorescence of Merino fibres was found to vary along the length of the fibre, from highly fluorescent tips to barely fluorescent roots. It would appear that the same species' are responsible for the fluorescence at the tip and root. An increased level of fluorescence was observed when the disulphide bonds of cystine were oxidised to cysteic acid residues, due to the removal of the quenching disulphide bond. Each of the morphological components examined (cuticle, cortex and cell membrane complex) were fluorescent. Chemical bleaching treatments caused an increase in the fluorescence intensity of the components, with the same emitting species being responsible as in the non-bleached.

Fluorescent lamps and laser irradiation were used to investigate the (different) effects of UV and blue light upon the fluorescence intensity of wool. UV irradiation caused a rapid increase in intensity which was accompanied by a definite protective effect being exhibited by the wool yolk. The enhancement of the white appearance in photobleached wool is not due to the production of UV absorbing species which exhibit blue fluorescence, but to the destruction of yellow coloured residues. Northlight fluorescent lamps led to no change in the level of fluorescence whereas blue laser light caused a decrease. The level of natural fluorescence decreased when wool, embedded in immersion oil or glycerol, was irradiated with UV light.

The effect of four reducing agents on wool was investigated: in the presence of UV irradiation, blue light irradiation and in the dark. The agents fell into two groups. The first group (Rongalit C and sodium hypophosphite) had little effect on their own but in the presence of irradiation retarded the effect of light. The second group (Blankit D and thiourea dioxide) had an effect on their own and upon irradiation acted as photosensitisers causing extensive photobleaching. The difference between the two groups was attributed to the pH of the solutions used. The whitening observed was (generally) due to the destruction of visible absorbing chromophores rather than to the production of UV absorbing chromophores which exhibit blue fluorescence.

Wool yolk is highly fluorescent. The amount of wool yolk/intensity of wool yolk fluorescence was found to vary along a Merino wool fibre from 'zero' at the tips to intense at the roots. The fluorescence of wool grease consists of two components - 'grease' and chlorophyll. The 'grease' fluorescence reveals the presence of a number of chromophores/components. An attempt to isolate these was only partly successful. Chlorophyll could be detected in all the wool greases examined. Weathering was found to result ultimately in a decrease in the fluorescence intensity arising from both the 'grease' and chlorophyll components. UV irradiation revealed that complex photochemical reactions were occurring.

The role of wool yolk in the photoyellowing of wool in nature was investigated. Using special conditions no migration of fluorescent probes (models for photosensitisers) into the wool structure could be observed. The 'swelling' solvent, toluene/methanol (9:1) was found to act as a photosensitiser.

PUBLICATIONS

The work contained in this thesis has so far resulted in the following publications:

1. The natural fluorescence of wool.
S. Collins, R.S. Davidson, P.H. Greaves, M. Healey and D.M. Lewis.
J. Soc. Dyers Col., 104, 348-352, (1988).
2. Probing the photochemistry of wool by microspectrofluorimetry.
S. Collins, R.S. Davidson, M.E.C. Hilchenbach and D.M. Lewis.
Poster presented at the XIIth IUPAC Symposium on Photochemistry,
Bologna, Italy, 1988.
3. Aspects of the photobleaching and photoyellowing of wool.
S. Collins and R.S. Davidson.
Accepted for publication by *J. Soc. Dyers Col.*

ABBREVIATIONS

A	absorbance
Ala	Alanine
AM	acceptor molecule
Arg	Arginine
Asp	Aspartic acid
BA	Buenos Aires
CIE	Commission Internationale de l'Eclairage
cmc	cell membrane complex
CSIRO	Commonwealth, Scientific and Industrial Research Organisation
Cys	Cystine
CySO ₃ H	Cysteic acid
D	donor molecule
DWI	Deutsches Wollforschungsinstitut
esr	electron spin resonance
EW	excitation wavelength
F	fluorescence
FTIR	Fourier transform infra-red (spectroscopy)
FWA	fluorescent whitening agent
Glu	Glutamic acid
Gly	Glycine
h ν_f	fluorescence
h ν_p	phosphorescence
His	Histidine
HPI	3 α -hydroxypyrrolidinoindole
hplc	high performance liquid chromatography
IC	internal conversion
Ile	Isoleucine
ISC	intersystem crossing
IWS	International Wool Secretariat
LCAO	linear combination of atomic orbitals
Leu	Leucine
Lys	Lysine
M	molecule
Met	Methionine
n	number of samples
NFK	N-formylkynurenine

OD	optical density
P	phosphorescence
Phe	Phenylalanine
PPI	3a-hydroperoxypyrrolidinoindole
Pro	Proline
PVA	poly(vinyl alcohol)
Q	quencher
rpm	revolutions per minute
s	standard deviation
S _n	singlet state
SDS	sodium dodecyl sulphate
sem	standard error of the mean
Ser	serine
T	transmittance
T _n	triplet state
Thr	Threonine
tlc	thin layer chromatography
Trp	Tryptophan
Tyr	Tyrosine
UV	ultraviolet
Val	Valine
VC	vibrational cascade
WRONZ	Wool Research Organisation of New Zealand
\bar{x}	mean
YI	Yellowness Index

CHAPTER 1

INTRODUCTION TO PHOTOCHEMISTRY

1.1 PREFACE

‘And God said, let there be light: and there was light. And God saw the light, that it was good’ Genesis 1.3,4

Photochemistry is concerned with the chemical and related physical effects of electronic excitation produced by the interaction of electromagnetic radiation (ultraviolet, visible and near infra-red) with matter [1]. Photochemistry is a very important area of study and many vital processes in nature involve photochemical reactions, such as photosynthesis and vision [2]. This reflects the fact that the major source of energy on earth is the Sun’s radiation.

Many textbooks covering photochemistry have been written [e.g. 1-11] and this chapter serves only to give a brief introduction to the subject and illuminate those areas which are of direct concern to the work presented in this thesis.

1.2 FUNDAMENTAL PRINCIPLES

1.2.1 Basic Laws of Photochemistry

There are two basic laws of photochemistry. The first, known as the Grotthus-Draper Law, states that:

Only that light which is absorbed by a system can cause chemical change.

The second law, known as the Stark-Einstein law, states that:

A molecule undergoing photochemical changes does so through the absorption of a single quantum of light.

The latter statement refers to the primary process of any reaction.

These laws do not necessarily have complete applicability under all circumstances. For example, with regard to the Stark-Einstein law, it is known that two-quanta absorption can be induced by the intense coherent radiation of laser beams.

1.2.2 The Beer-Lambert Law

The absorption of radiation occurs in accordance with the Lambert and Beer Laws. The Lambert Law states that the fraction of incident radiation absorbed by a transparent medium is independent of the intensity of incident radiation and that each successive layer of the medium absorbs an equal fraction of incident radiation. The Beer Law states that the amount of radiation absorbed is proportional to the number of molecules absorbing the radiation, that is the concentration of the absorbing species. These laws can be combined to give the Beer-Lambert law which can be represented by:

$$I = I_0 10^{-\epsilon c \ell} \quad \text{or} \quad \log_{10} \frac{I_0}{I} = \epsilon c \ell$$

where

- I is the intensity of the transmitted radiation
- I_0 is the intensity of the incident radiation
- ϵ is the molar extinction coefficient ($\text{l mol}^{-1} \text{ cm}^{-1}$)
- c is the concentration (mol dm^{-3})
- ℓ is the thickness of the absorbing layer (cm)

The quantity $\log_{10} \frac{I_0}{I}$ is commonly known as the optical density, OD, or

absorbance, A , and the quantity $\frac{I}{I_0}$ is known as the transmittance, T . The values

quoted for the molar extinction coefficient normally refer to the position of maximum absorption, as the coefficient depends on the wavelength, temperature and solvent, but not upon the concentration.

If there is more than one absorbing species present the law becomes:

$$I = I_0 10^{-\Sigma \epsilon c l}$$

where the summation $\Sigma \epsilon c l$ is for all the absorbing species. This can also be expressed as the sum of the individual absorbances:

$$A = A_1 + A_2 + A_3 + \dots$$

1.2.3 Quantum Yield

The Stark-Einstein law leads directly to the index of efficiency of photochemical reaction, namely the quantum yield, ϕ . This may be defined as:

$$\phi = \frac{\text{number of molecules undergoing the particular process concerned}}{\text{number of quanta absorbed}}$$

If, as is more usually the case, several competing processes operate following the uptake of light by a molecule, then:

$$\Sigma \phi \text{ (primary processes)} = 1$$

This is not necessarily true for chemical product formation where secondary processes, such as free-radical and recombination reactions, are possible. An extreme example is provided by the hydrogen-chlorine chain reaction where $\phi(\text{HCl})$ may be as high as 10^6 . Hence it is important when quoting a value for a quantum yield to specify the process to which the value refers.

1.3 MOLECULAR EXCITATION

Molecules are formed by the joining of atoms by chemical bonds. The simplest description of bond formation and the electronic structure of molecules is given by molecular orbital theory (for a more thorough treatment of this subject see, for example, [12]). Molecular orbitals are derived from the *linear combination of atomic orbitals* (LCAO). A given number of atomic orbitals always gives rise to the same number of molecular orbitals. Combination of two atomic orbitals, on different atoms, by overlap along the internuclear axis results in the formation of two molecular orbitals, a bonding orbital (either σ or π), and an antibonding orbital (σ^* or π^*). Orbitals not involved in the LCAO process are described as

non-bonding n-orbitals, the energies of which are generally between that of the highest bonding and lowest antibonding molecular orbital.

Two electrons are assigned to each molecular orbital such that their spins are paired and, in general, a molecule in its ground state has all of its electrons spin-paired in the bonding (and non-bonding) orbitals. Electronic excitation of a molecule results in the promotion of an electron from one molecular orbital to another of higher energy (Figure 1.1). $\sigma \rightarrow \sigma^*$ and $n \rightarrow \sigma^*$ transitions are of relatively high energy, often requiring wavelengths of excitation shorter than 200nm. This is inconvenient from a practical point of view to investigate (due e.g. to components of the atmosphere absorbing) and also, does not occur in nature. It is the alternative excitation processes ($\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$) that are responsible for the bulk of organic photochemical reactions. The wavelength of light associated with these two excitations is observed in accessible regions of the ultraviolet and visible part of the electromagnetic spectrum.

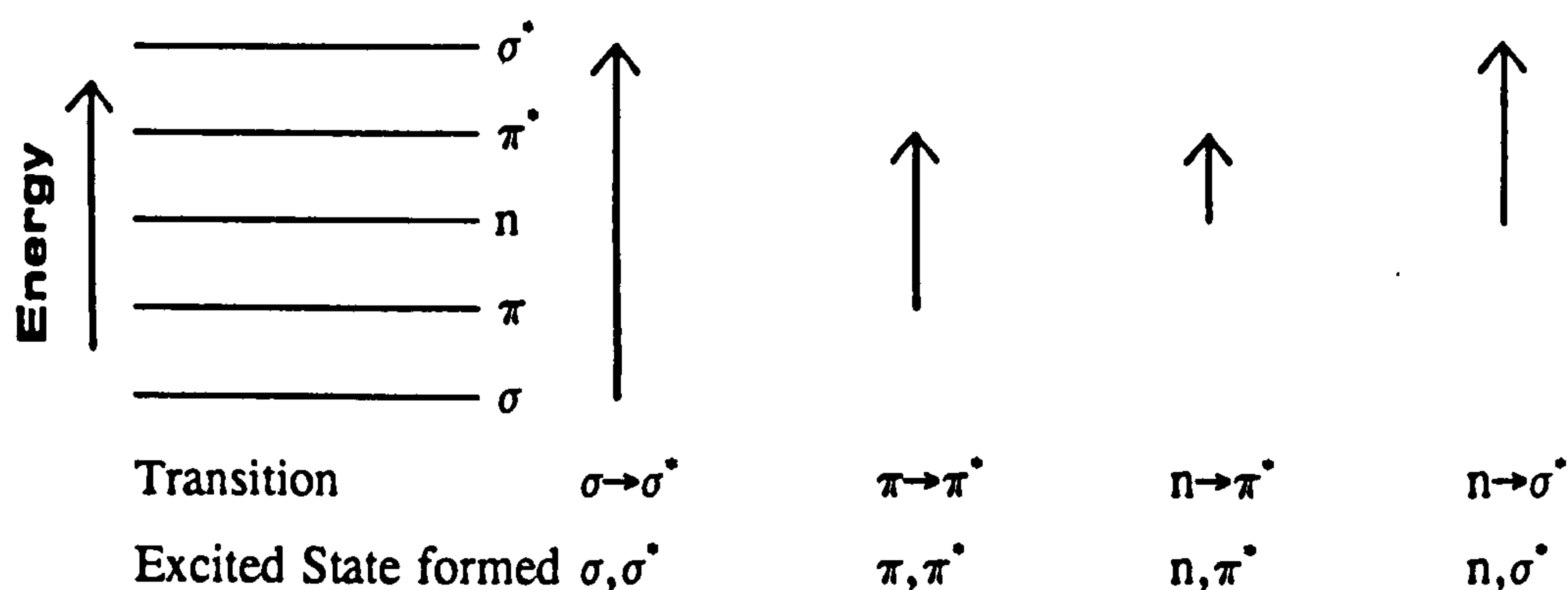


Figure 1.1 Electronic excitation processes in organic molecules

Once a molecule has been excited it contains two unpaired electrons. If the spins are opposed the state has a multiplicity of one, and is a singlet, while if the spins are parallel the state has a multiplicity of three and is a triplet. In almost all organic molecules the ground state is a singlet. The various singlet states can be designated, in order of increasing energy, S_0 , S_1 , S_2 etc., while the equivalent triplet states are T_1 , T_2 etc. Triplet states have lower energies than the equivalent singlet states. In addition, the triplet will have a longer lifetime than the singlet state since a spin inversion must accompany any deactivation of the triplet.

Electronic energy is not the only type of quantised energy possessed by a molecule. Unlike an atom, molecules can have vibrational and rotational energy, and it is possible that when a transition takes place there is a change not only in the electronic state but also in the vibrational and rotational states. This explains why the absorption spectra of molecules in solution consist of bands rather than lines.

1.4 DISSIPATIVE PATHWAYS

When a molecule has been photochemically promoted to an excited state, it does not remain there for long. The processes responsible for the physical dissipation of the excess energy may be differentiated and classified as in Figure 1.2.

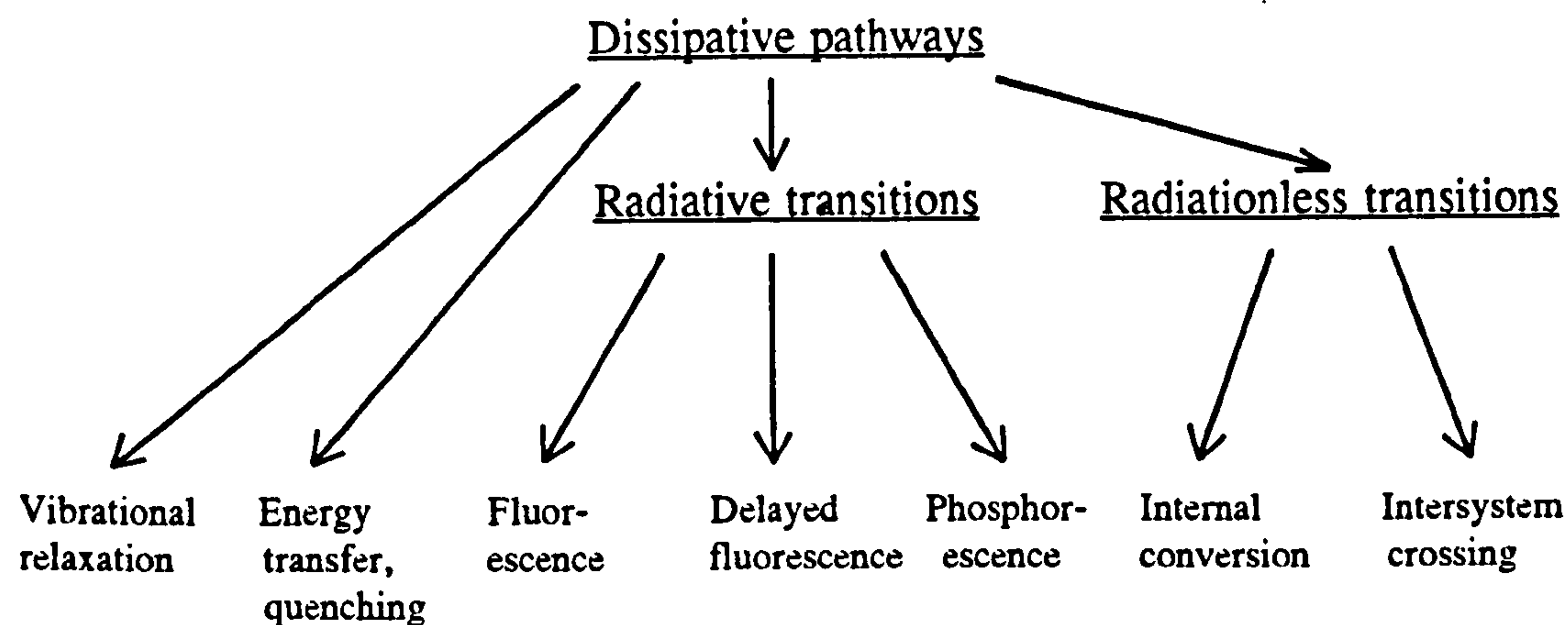


Figure 1.2 Physical pathways for the dissipation of electronic energy [2]

It should be realised that these processes can compete with each other for the deactivation of an excited state, and the relative magnitude of the rate constants determines the contribution made by a particular pathway. Most of these pathways are shown in a modified Jablonski diagram (Figure 1.3), after Jablonski [13].

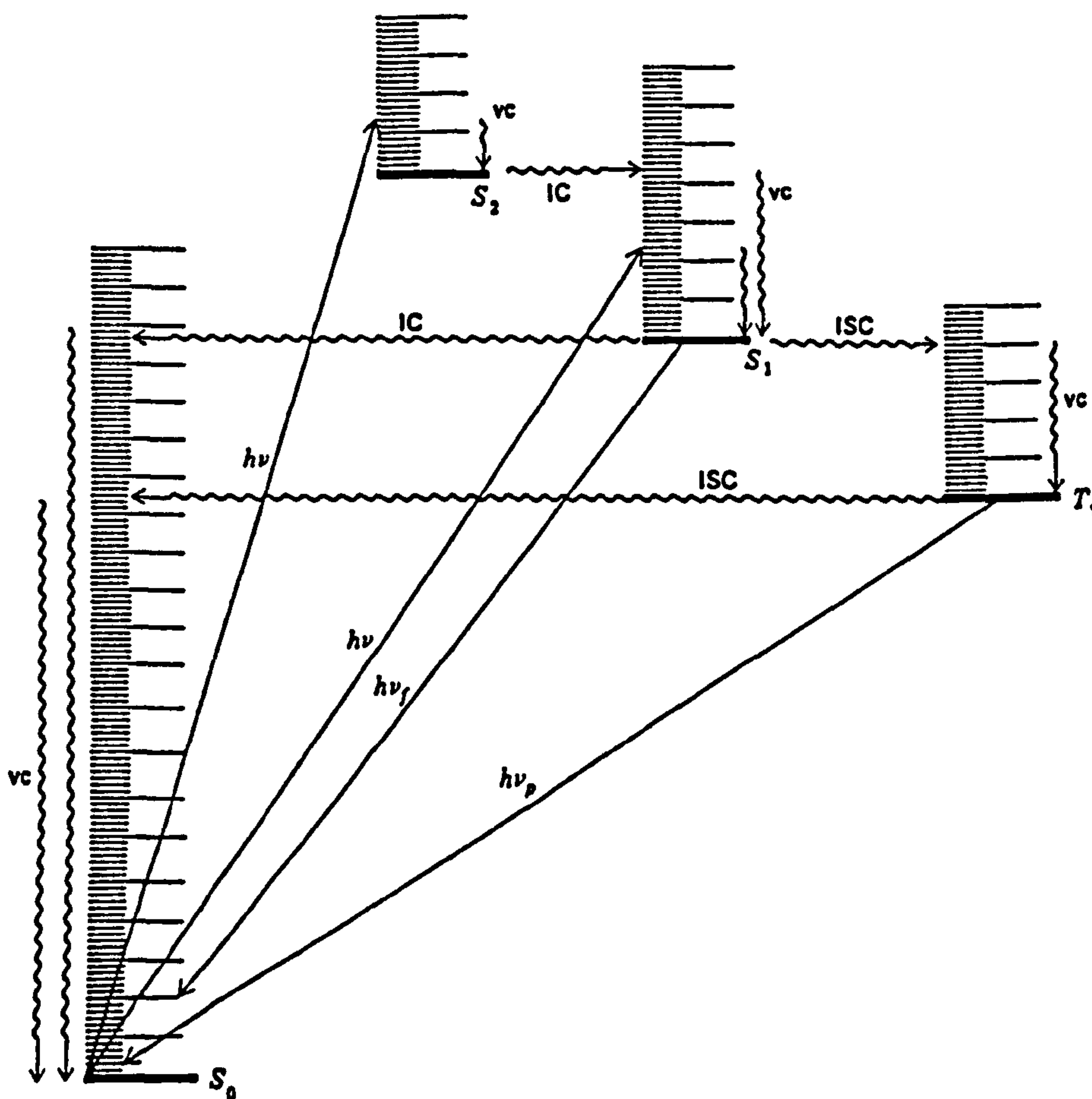


Figure 1.3 Modified Jablonski diagram showing transitions between excited states and the ground state. Radiative processes are shown by straight lines; radiationless processes by wavy lines. IC = internal conversion; ISC = intersystem crossing; VC = vibrational cascade; $h\nu_f$ = fluorescence; $h\nu_p$ = phosphorescence [11].

1.4.1 Vibrational Relaxation

Usually an excited species finds itself at the moment of its generation with excess vibrational (and rotational) energy in addition to the electronic energy. Loss of vibrational energy (called vibrational relaxation or vibrational cascade) is largely dependent upon collisions, as a result of which vibrational energy is converted into kinetic energy. At sufficiently high pressures, or in solution where the collision rate is of the order of 10^{13}s^{-1} , total vibrational relaxation is the rule and emission

occurs almost exclusively from the lowest vibrational level. After taking into account Internal Conversion and Intersystem Crossing (see Section 1.4.3) it means that S_2 and higher singlet states decay very rapidly to either the lowest excited singlet state (S_1) or the lowest triplet state (T_1).

It is possible for a molecule in the S_1 state to cascade down through the vibrational levels of the S_0 state. However, as the amount of energy is large the process is quite slow, so most molecules in the S_1 state adopt other pathways.

1.4.2 Radiative Transitions

In radiative transitions, represented by straight arrows on a Jablonski diagram (Figure 1.3), an excited species passes from a higher excited state to a lower one with the emission of a photon. Three processes may be distinguished:

1. Fluorescence ($h\nu_f$) This process, which generally happens within 10^{-9} s, involves a molecule in the S_1 state dropping to some low vibrational level of the S_0 state all at once by giving off the energy in the form of light. Not all molecules fluoresce. For those which do, the fluorescence emission spectra are usually the approximate mirror images of the absorption spectra. This occurs because the fluorescing molecules all drop from the lowest vibrational level of the S_1 state to various vibrational levels of S_0 , while excitation is from the lowest vibrational level of S_0 to various levels of S_1 . The only peak in common is the one resulting from transitions between the lowest vibrational levels of the two states. In solution, even this may be noncoincidental because the two states are solvated differently. Fluorescence nearly always arises from a $S_1 \rightarrow S_0$ transition, though azulene (Figure 1.4) and its simple derivatives are exceptions, emitting fluorescence from $S_2 \rightarrow S_0$ transitions.

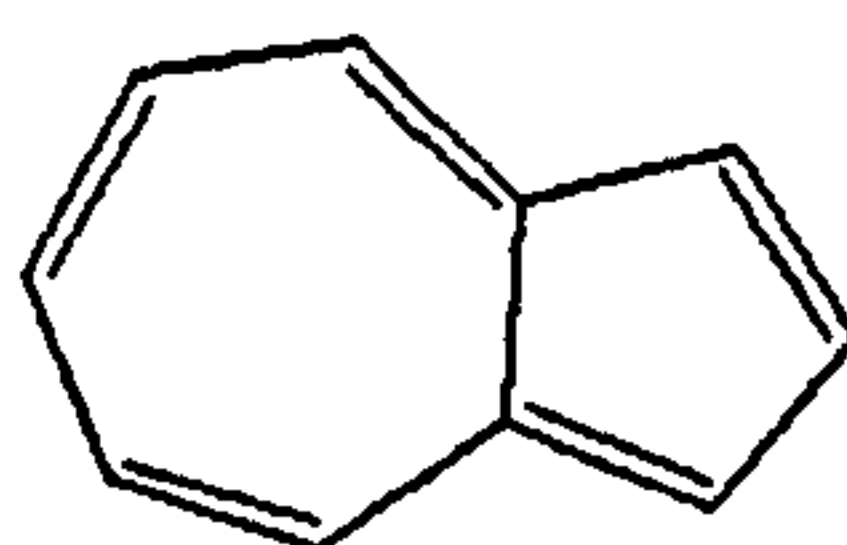


Figure 1.4 Structure of azulene

2. Phosphorescence ($h\nu_p$) This process involves a molecule in the T_1 state returning to the S_0 state by giving off the energy in the form of light. Phosphorescence is very slow ($\sim 10^{-3}$ to 10^1 s) due to the process being a spin forbidden transition. When fluorescence and phosphorescence occur in the same molecule, phosphorescence is found at lower frequencies (because of the higher difference in energy between S_1 and S_0 than between T_1 and S_0) and is longer-lived (because it is a forbidden transition).

3. Delayed (or slow) fluorescence ($h\nu_f$) This has the same wavelength as fluorescence but occurs much more slowly. One way in which delayed fluorescence arises is when a molecule in the T_1 state acquires enough thermal energy to raise it to a higher vibrational energy (equal in energy to the lowest S_1 level) and then crosses to S_1 , which of course can then fluoresce. Delayed fluorescence caused in this manner obviously increases with increasing temperature. Delayed fluorescence can also arise from a collision between two triplet molecules in which an excited singlet is produced (triplet-triplet annihilation) as well as by the combination of a radical cation with an electron or a radical anion.

1.4.3 Radiationless Transitions

Radiationless (or non-radiative) transitions occur between isoenergetic vibrational-rotational levels of different electronic states. Since, there is no change in the total energy of the system, no photon is emitted, and the process is represented by a horizontal line on a Jablonski diagram (Figure 1.3). Wavy arrows are used (e.g. $S_1 \rightarrow S_0$) to distinguish radiationless transitions from radiative ones. Two processes may be distinguished:

1. Internal Conversion (IC) This is a radiationless transition between isoenergetic states of the same multiplicity, e.g. $S_2 \rightarrow S_1$, $T_2 \rightarrow T_1$. Such transitions between upper states are extremely rapid, accounting for the negligible emission from upper states. Internal conversion from the first excited singlet state ($S_1 \rightarrow S_0$) is so much slower that fluorescence can compete.

2. Intersystem Crossing (ISC) This is a radiationless transition between states of different multiplicity, e.g. $S_1 \rightarrow T_1$. The radiationless deactivation of the lowest triplet ($T_1 \rightarrow S_0$) is a process in competition with normal phosphorescence. The intersystem crossing $S_1 \rightarrow T_1$ or $S_1 \rightarrow T_{>1}$, which is competitive with (and reduces the quantum yield of) fluorescence, is the process by which the triplet manifolds are normally populated. Since a singlet state usually has a higher energy than the corresponding triplet, this means that energy must be given up. One way for this to happen is for the S_1 molecule to cross to a T_1 state at a high vibrational level and then for the T_1 to cascade down to its lowest vibrational level. This cascade is very rapid (10^{-12} s). When T_2 or higher states are populated, they too rapidly cascade to the lowest vibrational level of the T_1 state. The transition $T_1 \rightarrow S_1$ requires thermal activation of T_1 and is the basis of one of the mechanisms leading to delayed fluorescence (Section 1.4.2).

1.4.4 Transfer of Excitation Energy - Sensitisation and Quenching

Excitation of a ground state molecule by energy transfer from another excited species is termed sensitisation; while a quencher is any substance which accelerates the decay of an electronically excited state. Quenching may occur by a variety of processes as illustrated in Figure 1.5. Quenching can be both intra- and intermolecular in nature.

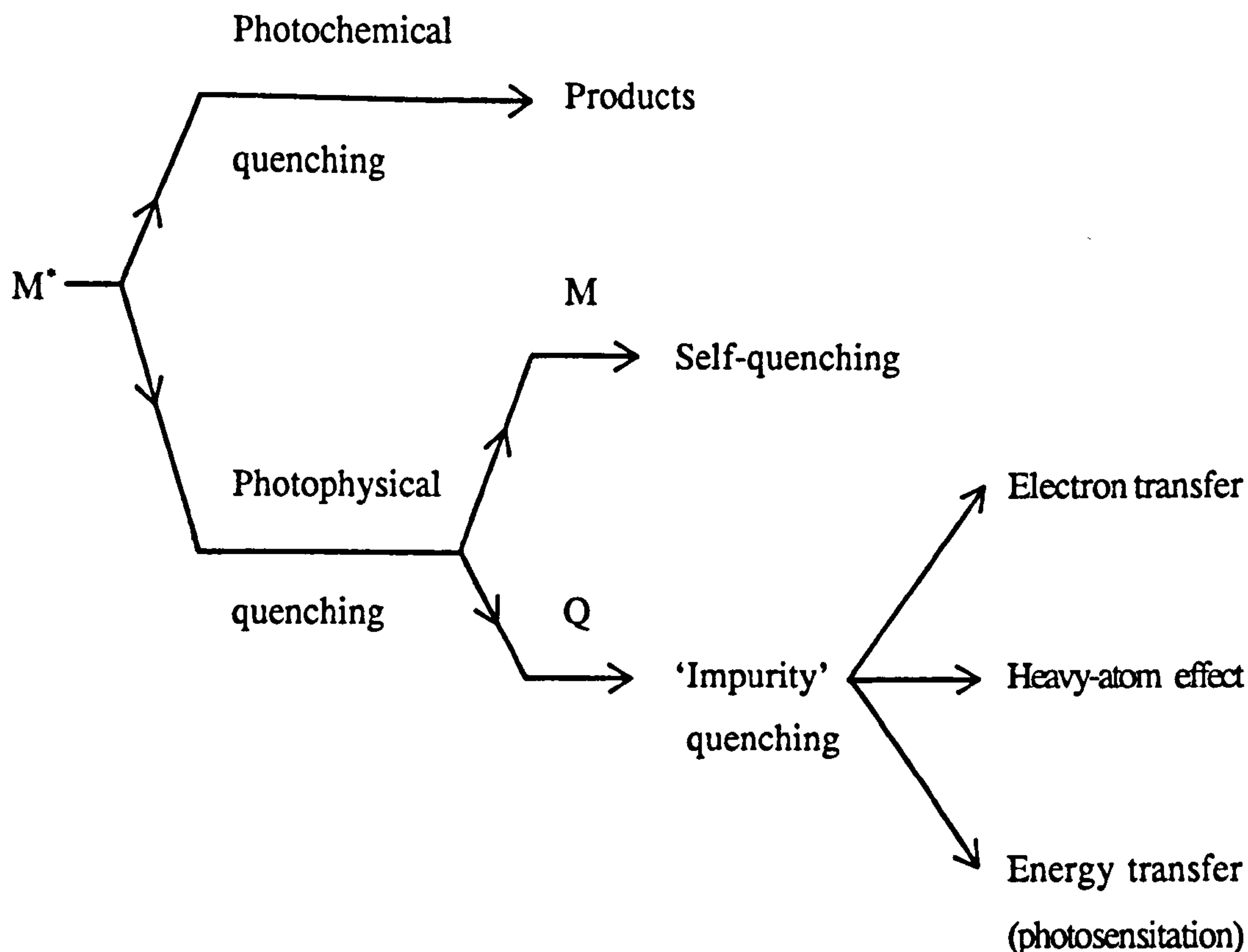
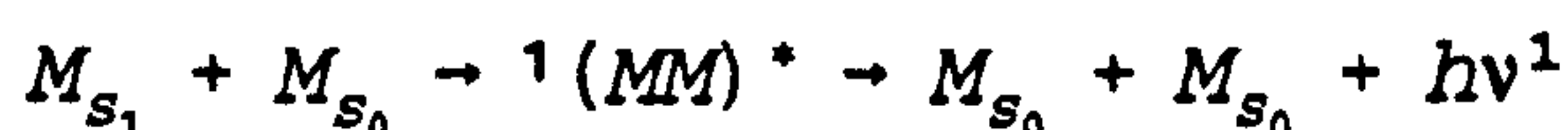


Figure 1.5 Quenching processes (where M^* is molecule M in an electronically excited state and Q is the quencher) [2]

Quenching by photochemical reaction forms the subject matter of organic photochemistry and is briefly discussed in Section 1.5. Photophysical quenching, which does not lead to new ground state products, can be divided into self-quenching, in which the quenching species is M , and 'impurity' quenching, where the quencher is some other chemical species.

Self-quenching can occur by triplet-triplet annihilation (see Section 1.4.2) and by concentration quenching. This is where an increase in the concentration of a solute is accompanied by a decrease in the intensity (quantum yield) of its fluorescence. Such quenching is often accompanied by the appearance of a new emission at longer wavelengths, the intensity of which increases with concentration. This arises from interaction of a molecule in its excited singlet state with a molecule in the same species in its ground state to produce an excited dimer or excimer:



where ${}^1(MM)^*$ represents the excimer and $h\nu^1$ is the longer wavelength fluorescence. Excimers exist only in the excited state, being dissociated and therefore undetectable in the ground state.

Three processes may be distinguished for 'impurity' quenching:

1. Electron Transfer Quenching This has been clearly established as an important process for the quenching of singlet excited states, but doubt exists as to the exact nature of the entities involved in the electron transfer. They may be exciplexes or encounter/collision complexes (Exciplexes $(MQ)^*$ are similar to excimers $(MM)^*$ except that the molecule is formed by the association of two different species. In encounter complexes $(M^{\bullet}\dots Q)$ the distance between partners is considerably larger than that occurring in excimers/exciplexes implying an ill-defined mutual orientation of the components, unlike excimers/exciplexes which occupy energy minima, and so have definite geometries). In polar solvents electron transfer is complete and leads to radical ions whereas in non-polar solvents electron transfer may be incomplete, giving rise to an exciplex which may relax either by fluorescence or radiationlessly by a 'return' of the partially transferred electron to regenerate M and Q in their S_0 or T_1 states. The latter may be thought of as an exciplex-induced internal conversion or intersystem crossing.

2. Heavy Atom Quenching Molecular fluorescence is quenched by the presence of species containing heavy atoms, and it seems that the phenomenon is due to the formation of a singlet exciplex (or encounter complex) which, because of the heavy atoms effect (an explanation of which is beyond the scope of this thesis), undergoes enhanced intersystem crossing to the triplet exciplex, followed by dissociation into its components:



since the exciplexes elude detection, what is observed in such systems is:



It should be recognised that intersystem crossing can occur in exciplexes, as in other systems, even in the absence of heavy atoms.

Heavy atom quenching can occur inter- and intramolecularly, e.g. by the addition of xenon to a solution of an aromatic hydrocarbon and by the substitution of hydrogen with halogens at the 1-position of naphthalene, respectively.

2b. Quenching by Oxygen (This is really a specific example of impurity quenching processes). The quenching of the excited states of many organic molecules by oxygen is diffusion - controlled. Quenching of singlet states seems to occur both by collisional energy transfer, generating singlet oxygen,



and by the spin-allowed catalysed intersystem crossing,



Each encounter of oxygen with an excited molecule leads to quenching making it essential in all quantitative work to reduce the concentration of dissolved oxygen to the smallest possible value.

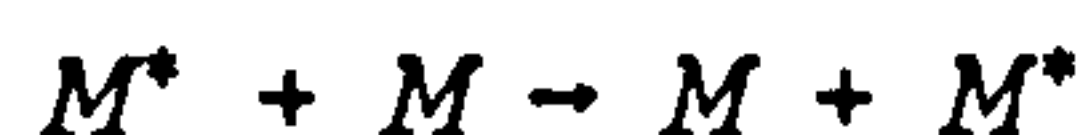
3. Electronic Energy Transfer In this phenomenon an excited donor molecule D^* collapses to its ground state with the simultaneous transfer of its electronic excitation energy to an acceptor molecule AM which is thereby promoted to an excited state.



It should be noted that the acceptor can itself be an excited state, as in triplet-triplet annihilation (see also Section 1.4.2).



What is observed in an energy transfer experiment is the quenching of the emission (or photochemistry) associated with D^* and its replacement by the emission (or photochemistry) characteristic of AM^* . Hence, although the photons are absorbed by D , it is AM which becomes excited. The processes resulting from AM^* generated in this manner are said to be sensitised. When the donor and acceptor are identical, the term energy migration is used, i.e.:



In the two most important types of photosensitisation, a triplet excited state generates another triplet, and a singlet generates a singlet. Both of these types can be useful for generating excited states when they are difficult to achieve by direct irradiation. Photosensitisation is most efficient when the donor D^* has a higher energy than the excited acceptor AM^* . The excess energy appears as kinetic energy of D and AM^* .

1.5 PHOTOCHEMICAL REACTIONS

Although both excited singlet and triplet species can undergo chemical reactions, bimolecular reactions are much more common for triplets, simply because these generally have much longer lifetimes. Excited singlet species, in most cases, have a lifetime of less than 10^{-10} s and undergo a physical dissipative process (Section 1.4) before they have a chance to react chemically. Therefore, photochemistry is largely the chemistry of triplet states.

The most common primary photochemical reactions are cleavage into free radicals, decomposition into molecules, and (in the presence of a suitable acceptor molecule) photosensitisation. Intramolecular rearrangement, photoisomerisation, hydrogen-atom abstraction and photodimerisation also occur.

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CHAPTER 2

INTRODUCTION TO WOOL

2.1 THE UNIQUE FIBRE

The term wool is applied to fibres from the fleece of the sheep [1]. Other animal hairs are used in textiles, for example, of the camel, alpaca, and llama, of the Cashmere and Angora (Mohair) goats, and of the Angora rabbit. These animal hairs are in limited supply, and very expensive. Wool is by far the most important animal fibre.

In terms of world fibre production, both natural and man-made, wool's share is small, at only 5% [2,3]. Cotton's share accounts for 48%, while synthetics contribute 37% and cellulose 8%. Flax only has 2%, while silk has less than 0.5% [3]. However, wool's unique properties make it far superior to these other fibres.

Wool provides great warmth for little weight by a variety of ways:

1. Its insulating quality. In a fabric this arises largely from the amount of still air trapped, first between the weave of the yarns, and second, between the fibres within the yarns [4]. This is amplified in wool by its natural 'crimp' which creates millions of microscopic air pockets throughout the fabric [5].
2. Fewer fibres touch the skin compared to other fabrics, so that less heat is conducted away from the skin [6]. Hence smooth cotton sheets feel cold while fleecy blankets feel warm.
3. The heat of absorption for wool is substantially greater than for any other fibre [7]. As the humidity of the air surrounding a wool fibre rises and falls, the fibre absorbs and desorbs water vapour. Heat is given out during the absorption phase, causing the temperature of the fibre to rise. Conversely, desorption results in a temperature drop. This is more likely to be perceived in garments normally worn against the skin.

4. While the surface of wool is water resistant [4,6] its interior is highly absorbent. Wool is the most hydrophilic of all natural fibres. Air-dry wool normally contains absorbed water equal to 16% of its weight (the standard regain) and wool can absorb 30% of its weight without feeling wet to the touch. (By comparison cotton absorbs 8%, synthetics usually less than 5%.)

The low water absorbency of synthetics allows the amount of moisture on the skin to increase, whereas the absorbency of wool increases with increasing water content. This greater ability to absorb more at greater moisture contents helps to keep the body cool (in summer) by the removal and evaporation of perspiration, so providing greater comfort. Wool is therefore also best for a hot climate. However, as wool keeps the water content of the wearer's skin constant it also means that the body feels less chilled in winter.

5. When wool absorbs water it causes the fibres to swell, making the fabric bulkier, decreasing air permeability, and lowering the wind-chill effect.

These points are best illustrated by the fleece on a sheep's back. In Britain, sheep which have a body temperature of 104°F (40.0°C), can carry snow on their fleeces without it melting showing that the fleeces of sheep keep the animals warm [4]. However, in the hot climate of Australia a fleece helps to keep the sheep cool. Here the air temperature rises above 107°F (41.7°C), although the temperature of the tip of the wool might reach 180°F (82.2°C).

The presence of water, plus the protein keratin, makes wool naturally flame resistant, requiring a higher temperature to ignite when compared with other natural fibres [6]. When aflame, it burns slowly, smouldering and charring but giving off little heat. A wool blanket is an effective way of smothering a flame. Many commonly used synthetics melt when burned unlike wool [5]. Fabrics which melt stick to the skin and cause serious burns.

The presence of water also means that wool does not offer the dry friction conditions which encourage the build-up of static electricity in clothes or carpets [5]. As a consequence, wool stays clean longer than other fabrics as it does not attract lint and dust from the air. Furthermore, wool's crimped fibres and their surface scale structure help keep dirt from penetrating the fibre.

Wool has good elastic recovery, giving it a springiness that makes clothes wrinkle resistant when dry and explains why wool felt covers piano hammers [6]. Wool can be bent 20,000 times without breaking [7]. By comparison, cotton breaks after 3,200 bends, silk after 1,800 bends and viscose after only 75 bends. Wool's high crimpiness enables it to absorb odours and noise in heavy machinery and stereo speakers.

2.2 MORPHOLOGY

Wool fibres consist of three morphological components - cortical cells, which make up the bulk of the fibre; cuticle cells, which surround the cortex; and the cell membrane complex, which acts as a glue bonding the cells together [8]. Coarse wool fibres have in addition a medulla, which consists of a series of specialised and vacuolated cells arranged along the axis of the fibre components [9].

Figure 2.1 shows schematically the morphological arrangement of the wool fibre components. It must be remembered that this is a generalized diagram of the wool fibre, as wool fibres can show great variation in diameter, crimp, and length, depending upon breed, diet, health and climate. Fibre diameter varies from approximately $16\mu\text{m}$ for the finest Merino wool to over $40\mu\text{m}$ for some coarse, long wool breeds [8].

Merino wool (64s quality, average diameter $20.6\text{-}22\mu\text{m}$) is most frequently used for chemical study, whereas coarser wools, such as Corriedale and Lincoln, are often used for physical studies because of their larger diameter which makes handling easier. Commercially, the fibre diameter has a major influence on the final end-use to which wool is put [2]. Fine wools are used for apparel while coarser wools are more suitable for carpets and hand knitting yarn. In the present work Merino wool was generally used.

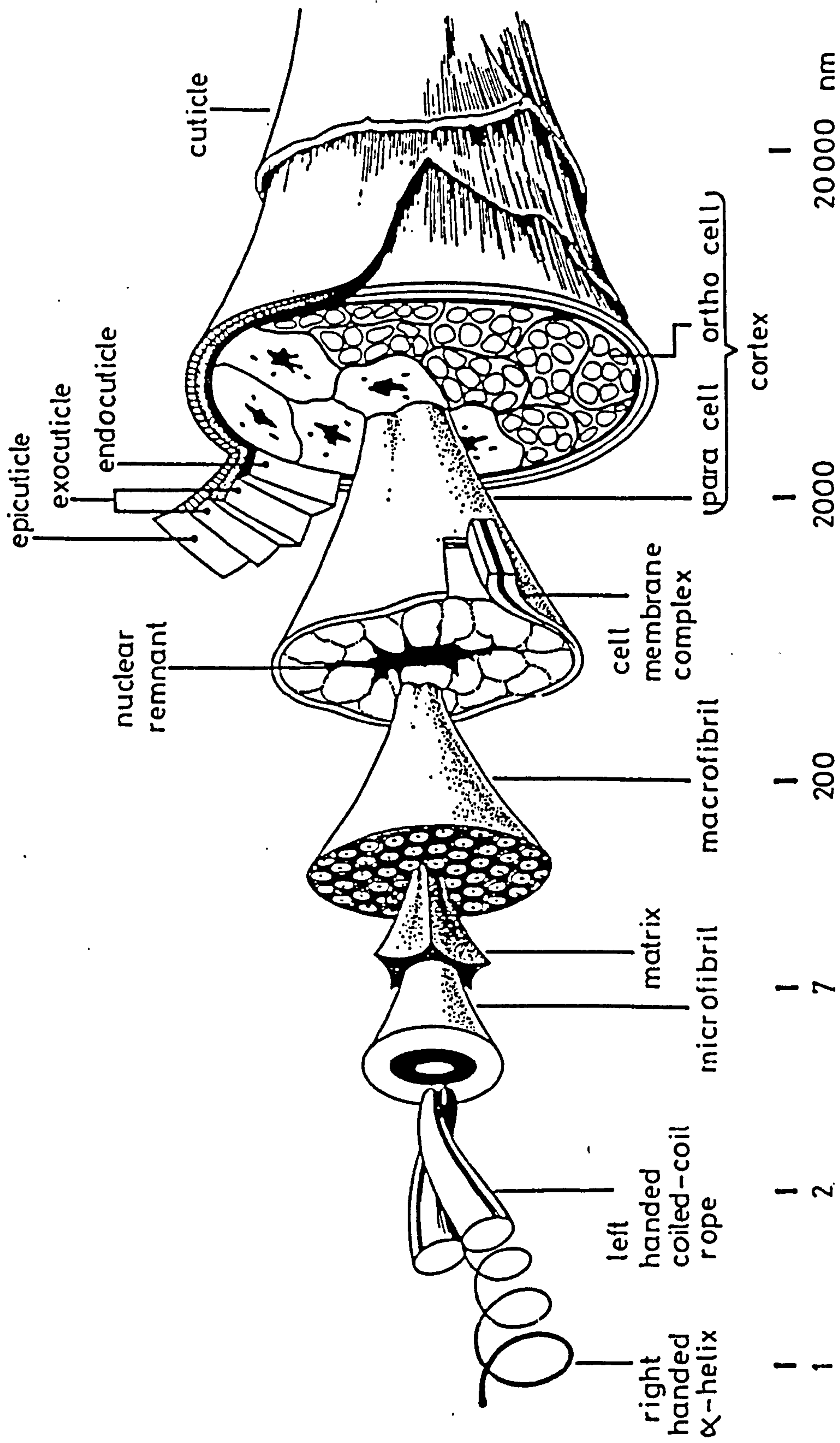


Figure 2.1 Structure of wool fibre [10].

2.2.1 Cuticle

Cuticle cells encase the cortex. The cuticle cells overlap each other like the tiles on a roof, the exposed scale edges pointing towards the fibre tip. They are responsible for the difference in the coefficient of friction of the wool fibre when measured in the with- and against-scale directions [8].

The cuticle of fine wool fibres is normally only one cell thick (except where cells overlap), whereas other keratin fibres such as human hair and pigs' bristle have been shown to have as many as 10 and 35 layers respectively [11,12].

Cuticle cells of Merino wool are generally rectangular, with dimensions of about $20 \times 30 \times 0.5 \mu\text{m}$ [13,14] and they represent about 10% by mass of the whole fibre [15]. Microscopic examination of fibre cross-sections shows that each cuticle cell is comprised of an enzyme-resistant exocuticle, and an enzyme-digestible endocuticle [16,17], surrounded by a thin hydrophobic membrane, the epicuticle [13].

2.2.2 Cell Membrane Complex

The flat overlapping cuticle cells are held to one another and to the cortical cells underneath by a cementing substance of non-keratinous material, known as the cell membrane complex [18]. This also surrounds individual cortical cells and is approximately 25nm thick. It is sometimes referred to as intercellular cement.

2.2.3 Cortex

Enclosed beneath the cuticle, in the interior of the fibre, is the cortex, which at about 90% by mass makes up the bulk of the wool fibre [8]. The cortex is generally differentiated into two parts, the orthocortex and the paracortex. The orthocortex is stained more heavily than the paracortex by basic dyes [19], acid dyes [20], and the salts of lead, mercury, silver and gold [21,23]. This differential staining allows easy identification by either light microscopy (detects dyes only) or electron microscopy (detects metal salts only). Fine wool fibres show bilateral

asymmetry; the paracortex is always located on the inside, and the orthocortex on the outside of the crimp wave. By contrast, Lincoln wool fibres (in common with most coarse, straight fibres) show cylindrical asymmetry, with an orthocortical core surrounded by a tubular paracortex.

Cortical cells are spindle-shaped, approximately $95\mu\text{m}$ long and $5\mu\text{m}$ across at the maximum width. They consist of highly organised rod-like microfibrils, sometimes called filaments (approximately $1\mu\text{m} \times 7.2\text{nm}$ diameter), packed in an amorphous matrix. The microfibrils occur in bundles, called macrofibrils, which are more tightly packed in orthocortical than in paracortical cells. Consequently the latter contain a higher proportion of matrix. Cortical cells also contain cytoplasmic debris (nuclear remnants), which are much more abundant in the paracortex than in the orthocortex.

2.2.4 Medulla

The medulla is situated near the centre of many coarse keratin fibres such as human hair, but it is not present in fine wool fibres [9]. It is formed from an axial stream of cells, the contents of which shrivel up during dehydration leaving a series of vacuoles along the fibre axis. While many variations occur in the shape and size of this part of the fibre it is believed to make little or no contribution to the chemical properties of the fibre.

2.2.5 Component Comparison

The typical dimensions of the morphological components of a Merino wool fibre are shown in Table 2.1. Wool fibres can show great variation depending on breed. Fibre length, based on annual shearing, varies from 30cm for coarse Cotswold wool down to 7-8cm for some fine Merino wool [8]. This does not seem very much, however as each Merino wool fibre grows 0.008 inch (0.2mm) a day [6], and there can be 60,000 wool follicles per square inch of skin and so a hundred million fibres in one fine Merino fleece, then one Merino can produce nearly 5,500 miles of wool fibre a year, at the rate of two-thirds of a mile an hour. The fibres of five Merinos, joined end to end, could tie a bow around the world!

Components	Length	Width	Diameter	Thickness
fibre	100 000	-	20	-
cuticle cell	30	20	-	0.5
cell membrane complex	-	-	-	0.025
cortical cell	95	-	5	-
macrofibril	10	-	0.3	-
microfibril	1	-	0.007	-

Table 2.1 Approximate dimensions of the components of a Merino wool fibre (μm) [8].

2.3 CHEMICAL COMPOSITION

Raw wool, when first shorn from the sheep's back, often contains less than 50% of clean fibre due to contamination by wool wax, suint, sand, dirt, and vegetable matter [8]. Together, wool wax and suint may comprise 20-50% of the weight of the raw wool (see Chapter 3).

2.3.1 Wool is not keratin only

Wool is known as an α -keratin [8]. However it is incorrect to think of wool as only containing keratin. In addition to 85% keratin, wool contains about 15% of other cell components, while pigmented and/or medullated wool contain even more of the non-keratinous constituents [24]. Table 2.2 illustrates this.

Although it is argued that it is unnecessary to consider the non-keratinous 15%, as the mechanical properties of a wool fibre can be explained on the basis of the keratin fibrils alone, [26,27] it is only the intact form of the biological composite structure of the keratin macrofibrils encased in the continuous network of the cell membrane complex, that guarantees the maintenance of the known chemical, physical and mechanical properties of wool [24].

Morphological Component	Percent in Fibre		Keratin	Other Constituents
Cuticle				
Exocuticle	6.4		6.4	
Endocuticle	3.6	<u>10</u>		3.6
Cell Membrane Complex				
Lipid	0.8			0.8
Soluble Proteins	1.0			1.0
Resistant membranes	1.5	<u>3.3</u>		1.5
Cortex				
Nuclear remnants and intermacrofibrillar material	12.6			12.6
Macrofibrils	74.1	<u>86.7</u>	74.1	
	100.0	100.0	80.5	19.5

Table 2.2 Stoichiometry of Merino wool [25]

Although the cell membrane complex, for example, only constitutes a small portion of the mass of the wool fibre there are many indications and suggestions that it has a major influence on many fibre and fabric properties [28]. Hence, it has been suggested that cell membrane complex components can affect mechanical properties such as abrasion resistance [29,30]; chemical properties such as susceptibility to attack by chemical finishing agents; and diffusion behaviour such as movement of dyes and other molecules into and within the fibre [18,24,31-34].

2.3.2 Amino Acid Composition

However, while wool is not only keratin it is mainly keratin, and on hydrolysis yields the 18 amino acids commonly present in the hydrolystates of most proteins (see Table 2.3) as well as some 'exotic' constituents such as citrulline and N_ε-[γ-glutamyl]lysine [39]. The relative amounts of these amino acids may vary from one wool sample to another, even within a single breed of sheep. Table 2.4 shows the amino acid composition of four different samples of Merino wool. Amino acid compositions can be expressed in a number of different ways and two samples for

each of the two commonest ways ($\mu\text{mol.g}^{-1}$ and mol.100mol^{-1}) are given. While some of the differences between the compositions may be due to experimental error, others are undoubtedly real.

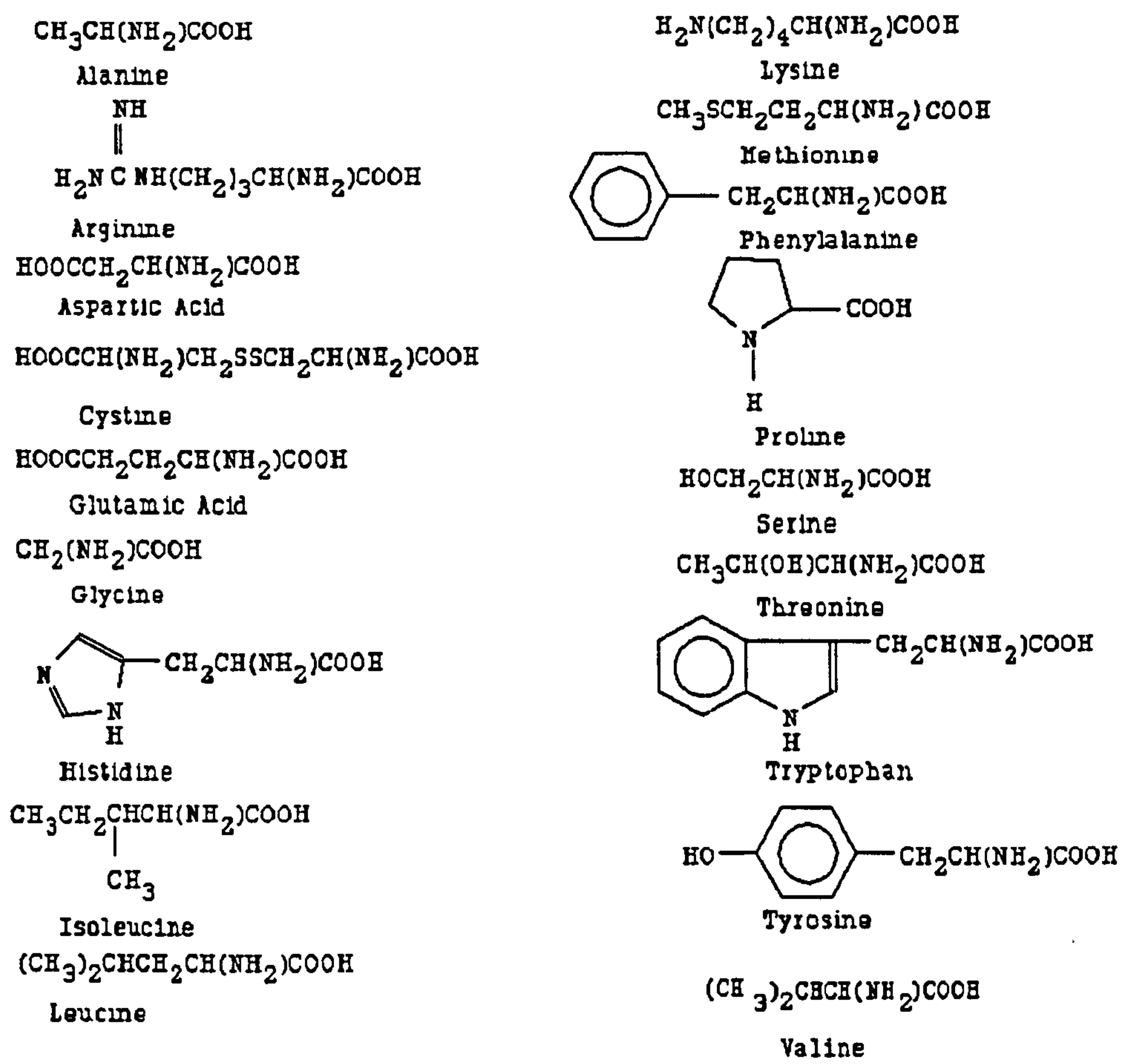


Table 2.3 Structures of amino acids.

Tryptophan is not included in Table 2.4 as it is destroyed under the conditions of acid hydrolysis used. It can be determined by amino acid analysis after hydrolysis with toluene-4-sulphonic acid in the presence of tryptamine [40], or with enzymes [41,42]. Values in the range 35-44 $\mu\text{mol.g}^{-1}$ have been obtained [41-43]. Colorimetric analysis of partial acid hydrolysates gives less reproducible results [8].

The cystine contents of different wools range from 400 to 500 $\mu\text{mol.g}^{-1}$ whereas cysteine contents are much lower and are generally 20-40 $\mu\text{mol.g}^{-1}$ [8].

Amino Acid	Composition ($\mu\text{mol.g}^{-1}$)		Composition (mol.100mol^{-1})	
	Merino 64s [35]	Merino 64s [36]	Merino 64s [37]	Merino Wool [38]
CySO ₃ H	-	-	0.2	0.2
Asp	503	560	6.5	6.8
Thr	547	572	6.4	6.6
Ser	860	902	10.8	11.5
Glu	1020	1049	12.0	12.4
Pro	633	522	7.0	7.3
Gly	688	757	8.9	9.0
Ala	417	469	5.4	5.8
Val	423	486	6.0	5.4
Cys	472	461	6.2	5.5
Met	37	44	0.5	0.4
Ile	234	275	3.2	2.8
Leu	583	676	7.5	8.0
Tyr	353	349	4.1	4.0
Phe	208	257	2.8	3.0
Lys	193	269	3.2	3.0
His	58	82	0.9	0.8
Arg	602	600	8.2	7.4

Table 2.4 Amino acid composition of Merino wool

Changes in the diet of a sheep may influence the amino acid composition of wool, the cystine content (and thus the sulphur content of the wool) being especially susceptible to variation. For example, there is a seasonal variation in the wool from Romney Marsh sheep, the sulphur content being lowest when wool production is greatest and vice versa [44].

2.3.3 Terminal Amino Acid Residues

Pure single-chain proteins are characterised by a single N-terminal amino acid residue. In most cases this may be identified as a 2,4-dinitrophenyl derivative by treating the protein with 1-fluoro-2,4-dinitrobenzene, and then subjecting it to acid hydrolysis. Subjecting wool to this gives eight different N-dinitrophenylamino acids all of which are present in small amounts [45], and in most wools total less than $20\mu\text{mol.g}^{-1}$.

Other N-terminal amino acids residues in wool occur as N-acetyl derivatives. The amount of acetic acid ($50\mu\text{mol.g}^{-1}$) released on acid hydrolysis of wool greatly exceeds the total content of the free N-terminal amino acids [46]. Thus more N-terminal residues occur as N-acetyl residues than occur free.

Six C-terminal amino acid residues of wool have been determined by hydrazinolysis [47,48], which releases the C-terminal residues as free amino acids, but converts all others to the corresponding hydrazides. The small contribution (approximately $10\mu\text{mol.g}^{-1}$) which these residues make to the acidic character of wool is far outweighed by that of the side-chain carboxyl groups.

2.3.4 Basic and Acidic Side-Chains

Wool, like other proteins, contains both basic and acidic groups, and is therefore amphoteric. Table 2.5 shows the structure and approximate amounts of the basic and acidic residues present in wool.

Wool's basic character is due predominantly to the side-chains of the arginine, lysine, and histidine residues present, rather than to the relatively small number of free N-terminal amino acid residues. All of these basic groups can attract acid dyes to wool. Additionally the side-chains of lysine and histidine residues are important sites for the covalent attachment of reactive dyes.

Structure	Residue	Amount ($\mu\text{mol.g}^{-1}$)
acidic residues		
$ \begin{array}{c} \text{-NH} \\ \\ \text{CHCH}_2\text{CO}_2\text{H} \\ \\ \text{-CO} \end{array} $	aspartic acid	200
$ \begin{array}{c} \text{-NH} \\ \\ \text{CHCH}_2\text{CH}_2\text{CO}_2\text{H} \\ \\ \text{-CO} \end{array} $	glutamic acid	600
$ \begin{array}{c} \text{-NH} \\ \\ \text{CHR} \\ \\ \text{CO}_2\text{H} \end{array} $	C-terminal	10
basic residues		
$ \begin{array}{c} \text{-NH} \qquad \qquad \text{NH} \\ \qquad \qquad \parallel \\ \text{CHCH}_2\text{CH}_2\text{CH}_2\text{NHCNH}_2 \\ \\ \text{-CO} \end{array} $	arginine	600
$ \begin{array}{c} \text{-NH} \\ \\ \text{CHCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{NH}_2 \\ \\ \text{-CO} \end{array} $	lysine	250
$ \begin{array}{c} \text{-NH} \\ \\ \text{CHCH}_2\text{-} \begin{array}{c} \text{NH} \\ \diagup \quad \diagdown \\ \text{C} \\ \diagdown \quad \diagup \\ \text{N} \end{array} \\ \\ \text{-CO} \end{array} $	histidine	80
$ \begin{array}{c} \text{NH}_2 \\ \\ \text{CHR} \\ \\ \text{-CO} \end{array} $	N-terminal	10

Table 2.5 Acidic and basic amino acid residues in wool [8]

There is an approximate balance between the acidic and basic groups in wool (see Table 2.5). Disturbance of the balance may lead to changes in properties such as tensile behaviour, settability and supercontraction [49-52]. The changes are due predominantly to the disruption of electrostatic interactions in the fibre, although changes in hydrogen bonding and hydrophobic interactions are also involved.

2.3.5 Crosslinks

The variety of bonds and interactions which exist between the protein molecules in wool fibres are responsible for maintaining the stable structure of the fibre and explaining many of its mechanical and chemical properties.

2.3.5.1 Noncovalent bonds

Noncovalent bonds are present in the keratin fibre stabilizing the α -helical structure of the polypeptide chains. These bonds can link different side-chain groups together or link side-chain groups within the protein-chain backbone. Three different types of noncovalent 'bonds' exist in keratin fibre:

1. Hydrogen bonds. A large number of hydrogen bonds occur in wool. For example, between carbonyl and amino groups within an α -helical chain, but these cannot be classified as crosslinks.

2. Hydrophobic interactions [53,54]. These are entropic interactions between non-polar side chain residues surrounded by water and are responsible for the tendency of these residues to avoid contact with the aqueous phase and to adhere to one another in the form of an intramolecular micelle.

The importance of the hydrophobic bonds becomes apparent when it is realised that the content of amino acids with non-polar side chains accounts for nearly half of the residues present in wool.

3. Ionic bonds (or salt linkages). These are formed by the mutual attraction of positively and negatively charged groups of the polypeptide chains. For example, between the carboxyl group of a glutamyl residue and an amino group of a lysyl residue as shown in Figure 2.2.

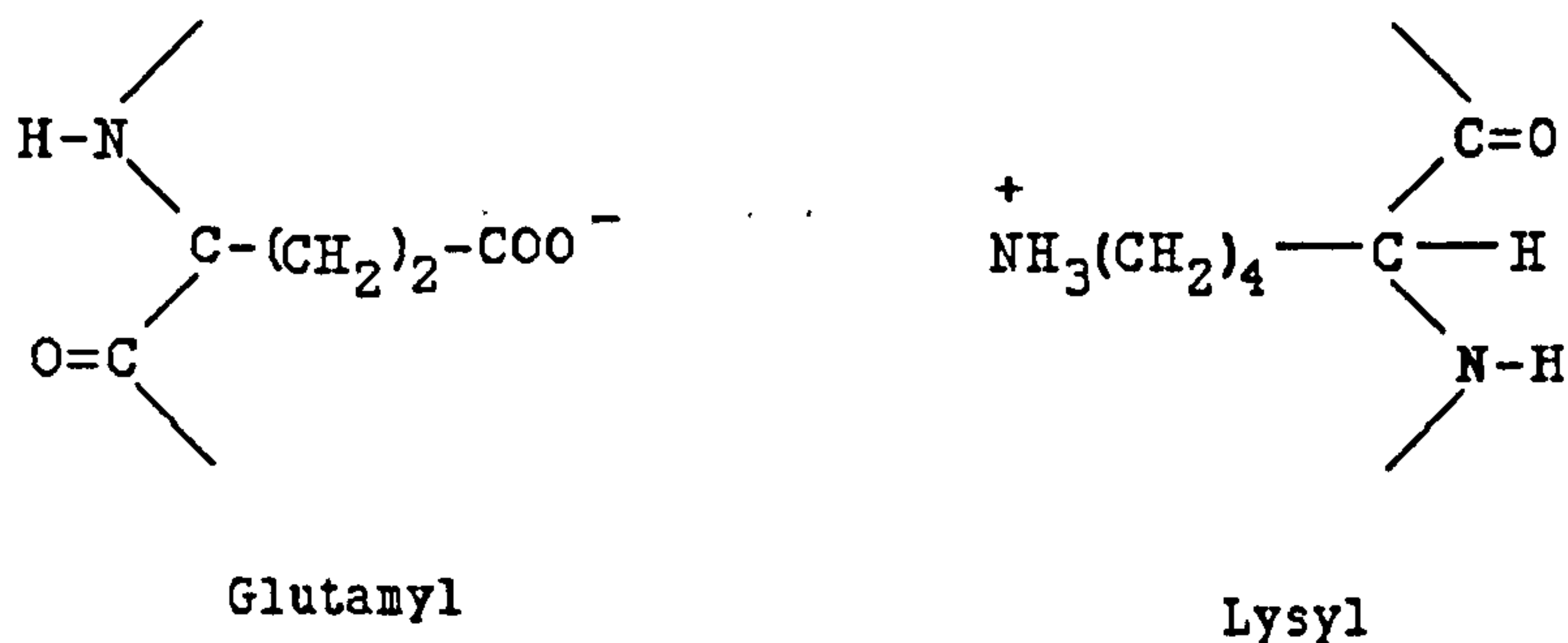
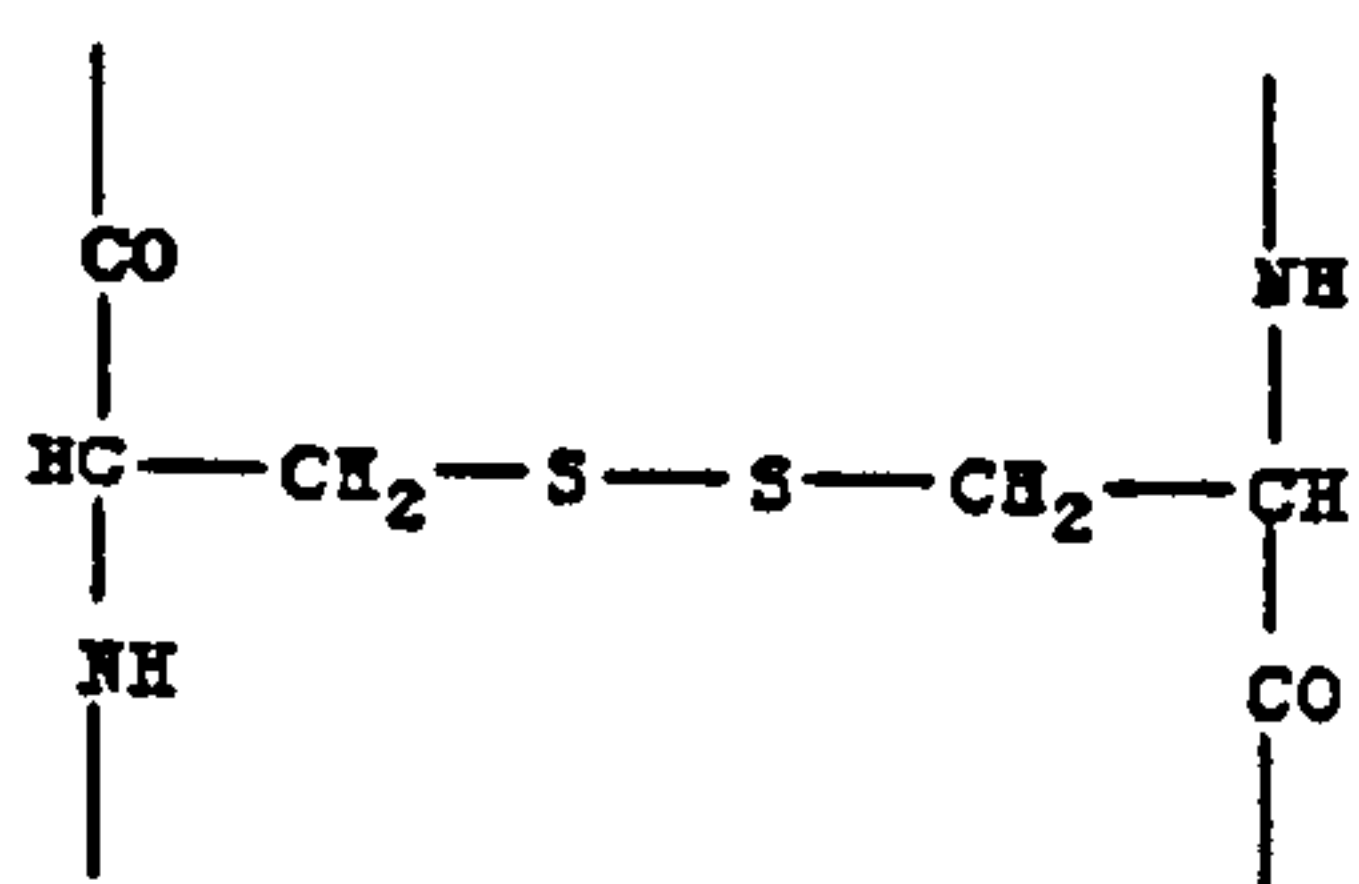


Figure 2.2 Ionic bond between glutamyl and lysyl residues.

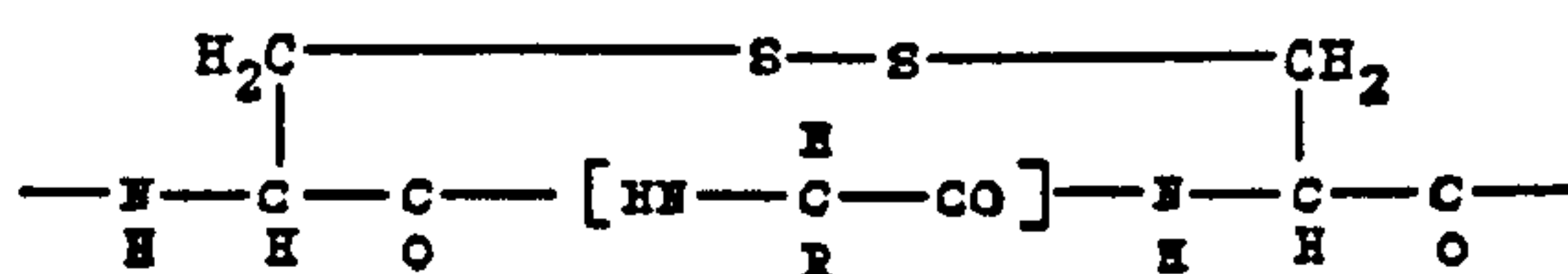
2.3.5.2 Covalent bonds

Three different types of covalent bonds exist in wool, including the most important crosslink of the disulphide bond.

1. Cystine crosslinks. This is the most likely distributed of all known crosslinks and plays an important role in stabilizing the wool fibre. Cystine allows two chains (or a single folded chain) to be covalently linked together via a disulphide bridge (Figure 2.3).



Interchain Disulphide Bond



Intrachain Disulphide Bond

Figure 2.3 Covalent disulphide bonds.

The disulphide groups, especially those which bridge half-cystine residues in different protein chains, retard conformational changes of wool proteins and are thus largely responsible for the relatively high wet strength of wool and its low lateral swelling and insolubility [8].

Although the sequences of several of the proteins extracted from wool after solubilisation using reduction/carboxymethylation are now known, the precise pairing of the original half-cystine residues of these proteins in the fibre is not. Many of the original disulphide crosslinks in wool must be interchainic, in order to account for the fibre's unique properties. It has even been suggested that all of the polypeptide chains in wool may be crosslinked, one to the next, so that the whole wool fibre is one giant molecule [55]. Although the disulphide crosslinks in a dry fibre are apparently stable, they readily rearrange under the influence of heat and water if the fibre is under stress. This facilitates conformational rearrangement of the wool proteins, and so leads to the relaxation of molecular stress in the fibre.

2. Isopeptide crosslinks. The isopeptide crosslink is a peptide bond joining the ϵ -amino group of a lysine residue to the γ -carboxyl group of a glutamic acid residue or, less commonly, to the β -carboxyl group of an aspartic acid residue.

Isopeptide crosslinks are not hydrolysed by the proteases used to prepare complete enzymic hydrolysates of wool, and thus their existence in proteins can be established by the isolation of either ϵ -(γ -glutamyl)lysine or ϵ -(β -aspartyl)lysine from enzymic hydrolysates (Figure 2.4). Estimates for the amount of ϵ -(γ -glutamyl)lysine in untreated wool range from 3 to 15 $\mu\text{mol.g}^{-1}$ [8].

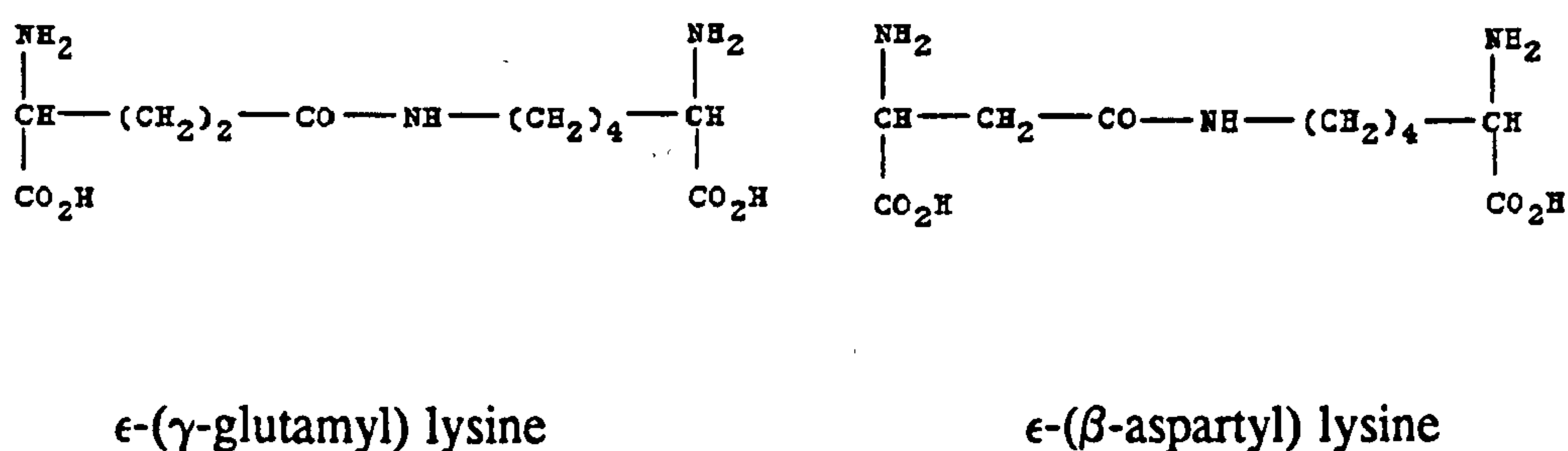


Figure 2.4 Isopeptide crosslinks.

3. Dityrosine and Trityrosine. Dityrosine is composed of two tyrosine residues which are linked by an ortho, ortho biphenyl linkage. Dityrosine and possibly the trimer, trityrosine, (Figure 2.5) have been isolated from wool hydrolysates in small amounts (2 to 3 $\mu\text{mol.g}^{-1}$), [56] and thus tyrosine residues may also participate in the crosslinking of wool.

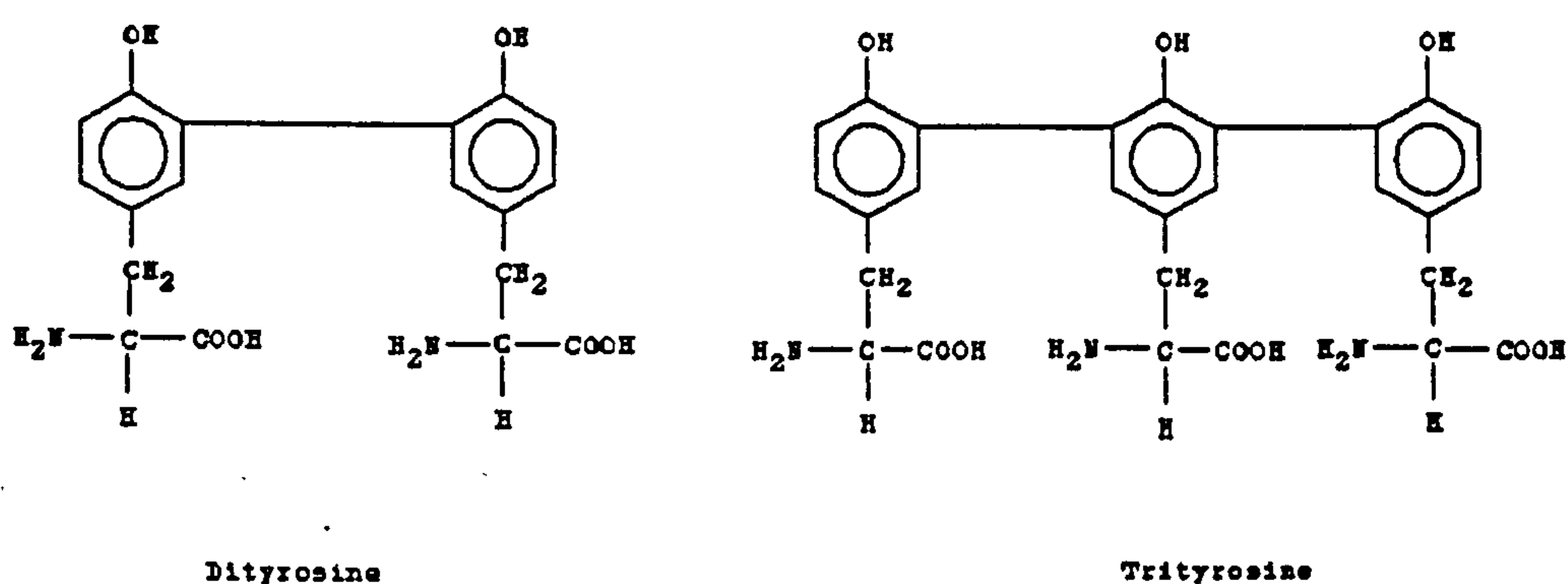


Figure 2.5 Structure of di- and trityrosine.

2.4 EFFECT OF LIGHT IRRADIATION ON WOOL

The exposure of wool to sunlight results in a number of physical and chemical changes. The most sensitive change is a discoloration resulting from the formation of yellow photodegradation products. Other changes which become evident after more prolonged exposure to sunlight include the loss of tensile strength and abrasion resistance (phototendering) and altered dyeing properties. In the present work phototendering has not been studied.

2.4.1 Early Investigations

Löbner in 1890 [57] appears to be the first to have commented on the fact that exposure to light alters the properties of wool. Rechberg in 1913 [58] noticed that cloth consisting of a mixture of dyed and undyed wool became darker on exposure to light. He attributed the change to destruction of those fibres unprotected by dyestuff, and patented a chroming process claimed to reduce the rate of damage.

Sauer in 1916 [59] was the first to indicate that the damage was of a chemical nature by showing an increase in 'soluble nitrogen' in wool following irradiation; an effect which was hindered by various dyestuffs. Kertesz in 1919 [60] demonstrated a decrease in strength and elasticity on materials exposed to natural weathering influences for eight months. He found that these changes were associated with acid production, an increase in solubility, and an increased affinity for methylene blue. He claimed that light was the most important atmospheric factor as he was unable to duplicate the effects of natural weathering by exposure to ozone, while damage produced by exposure to ultra-violet light gave results similar to atmospheric effects.

von Bergen in 1923 [61] carried out the first thorough investigation of the effects of light on wool. In a study of 'tippy' or unlevel dyeing of loose wool he found that while samples from the back and shoulder of the sheep dye unlevel, those from the belly dye uniformly. He concluded that the difference was due to the lack of exposure to direct sunlight of the belly wool. This was confirmed by exposing staples which dyed evenly, to sunlight under artificial conditions, and finding that the effect was duplicated.

2.4.2 UV Absorbance

The UV absorption of a Merino wool fibre, measured on a microspectrophotometer, is shown in Figure 2.6. Between wavelengths of 250 nm and 300 nm the absorption is essentially due to the presence of the amino acids tyrosine and tryptophan with minor contributions from cystine and phenylalanine. An absorption curve due to the amino acid composition of wool (Figure 2.6) can be

calculated from the amino acid composition of wool and the absorbance curves of tyrosine, tryptophan, cystine and phenylalanine in polymer films. In the 250-290 nm wavelength region the agreement between measured and estimated absorbances of wool is within 10%. This is acceptable in view of the computation of the estimated absorbances, the errors involved in amino acid analysis and the heterogeneity of wool fibres.

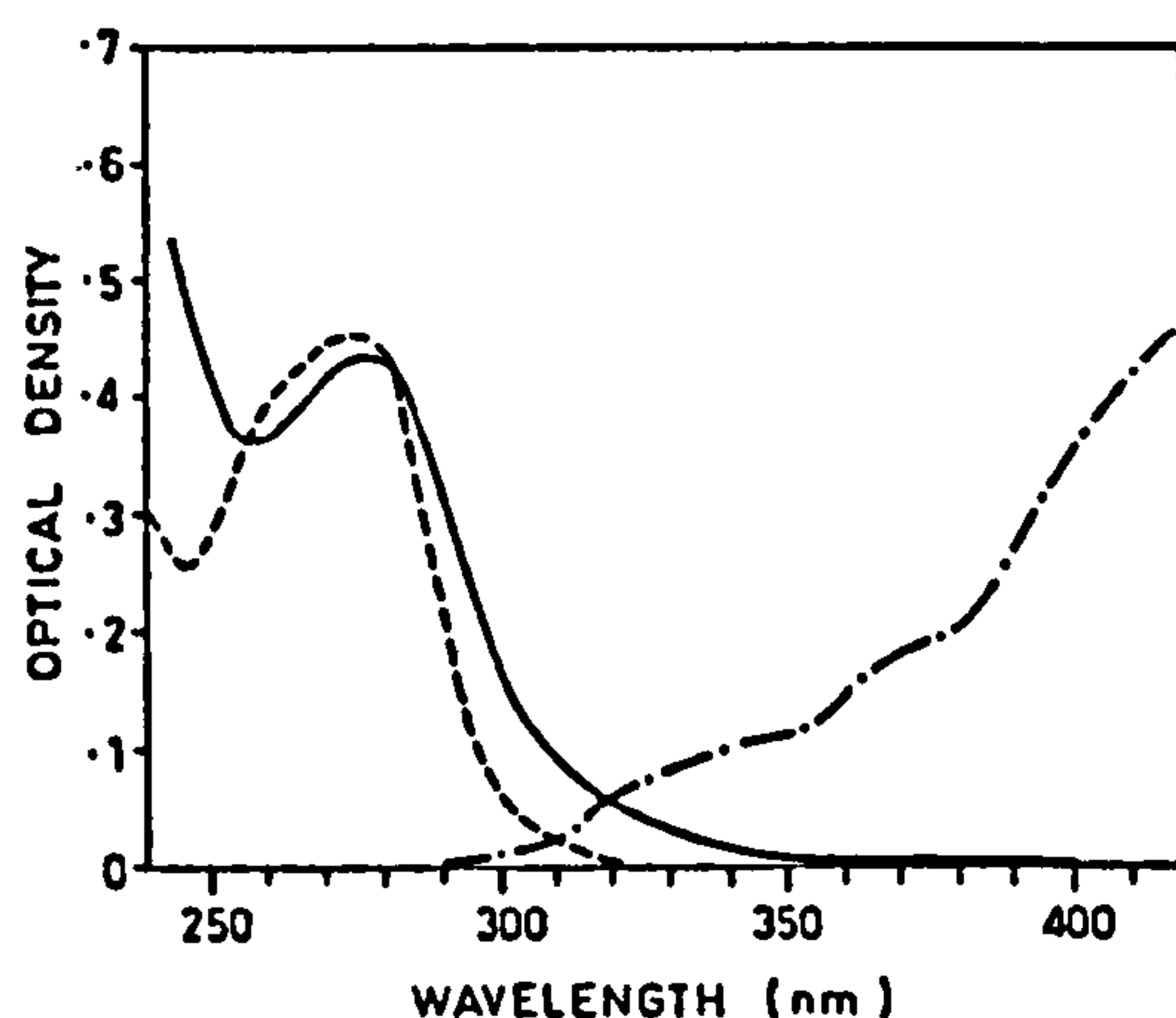


Figure 2.6 UV absorption spectrum of wool [62]. The ultraviolet absorption spectrum of a 6 μ m radial section of Merino-wool keratin (—), the absorption spectrum calculated from the amino-acid composition (---), and the relative intensity of Sydney south light at noon (-·-·-).

In the region above 290 nm the wool fibre has a much higher absorbance than the absorption by the amino acids predicts, indicating the presence of other absorbing species. The species causing this increased absorption have not yet been identified although it has been suggested that it may be due to the presence of photodecomposition products of wool arising from sunlight exposure during growth [63] or to the presence of natural pigment precursors [64].

The amount of energy in the wavelength region of sunlight below 300 nm is much less than in the 300-380 nm region. Although the absorption by wool in this higher wavelength region is relatively small, it is nevertheless important. This is demonstrated by the action spectrum for wool yellowing (see Section 2.4.3.2). This is different from the absorption spectrum of wool showing a gradual increase in yellowing with decreasing wavelengths below 380 nm. If the action spectrum

is corrected to correspond with the relative amounts of energy at different wavelengths in a typical sunlight spectrum reaching the Earth (see Figure 2.6) the maximum yellowing is found to occur in the 340-350 nm wavelength region [65].

2.4.3 Colour Change

2.4.3.1 Determination of Colour

As already mentioned (Section 2.4) the exposure of wool to sunlight results in a discoloration as a result of the formation of yellow photodegradation products. Consequently, it is necessary to be able to quantify such changes. Visual judgements may be used but are open to observer bias and suffer from lack of precision. For this reason attempts have been made to derive formulae for whiteness or yellowness.

One widely adopted parameter for assessing wool colour is Yellowness Index (YI) [66]. This originally [67] took the form:

$$YI = \frac{100 (A-B)}{G}$$

where A,B and G are reflectance values for amber, blue and green filters. A similar formula [68] based on CIE tristimulus values, X,Y and Z has also been used.

$$YI = \frac{100 (X-Z)}{Y}$$

The Yellowness Index decreases with improving observed whiteness. The difficulty with indices of yellowness is that they quantify yellowness only and it is possible for a white wool and a less white but grey wool to have the same yellowness index value.

Since colour is a three dimensional parameter, the description of wool colour with a single number is not wholly satisfactory. It is possible to use reflectance and absorption spectra [69] to give an indication of colour change but in reality they

do not measure colour as they do not take account of eye sensitivity. The CIE tristimulus values seek to overcome this. The Y-Z value can also be used as an indication of yellowness, and the Y value as an indication of brightness [70].

In the present work CIE tristimulus values have been used, as well as Yellowness Index measurements, according to the formula [71]:

$$YI = \frac{100 (1.316 X - 1.164 Z)}{Y}$$

2.4.3.2 Spectral Colour Change

Numerous studies have observed the effect of light on the colour of wool. With sunlight, yellowing [72-74] and bleaching [75-80] have been reported. Likewise with electrical sources both yellowing [72,74,78,81,82] and bleaching [81,82] have been seen. These conflicting results were found to be explainable upon a wavelength basis [83-91]. This is best illustrated by action spectra [83,88-91] and reflectance spectra [85,86], all of which differ at least slightly from each other. Often this includes differing relative YI values as well as wavelength ranges.

Three action spectra are shown in Figures 2.7-2.9. Yellowing is caused by UV light below 380 nm and increases with decreasing wavelength. The visible region of the spectrum, however, produces photobleaching. This information is reproduced in essence in the reflectance spectra of Figure 2.10. Additionally, for the 254 nm irradiation, it shows the orange absorber, which together with the violet-blue absorber, causes the wool to have a green colour. This type of discoloration cannot occur in sunlight because no wavelengths below 290 nm reach the earth's surface [8]. Hence, care should be taken in applying action spectra such as Figure 2.7, as the seemingly insignificant effect of visible light is realised to be not unimportant.

When wool is exposed to mixed radiation, notably sunlight, yellowing and bleaching proceed simultaneously, the overall effect being determined partly by the relative energies of the UV and blue-light regions of the spectrum [90]. Thus,

different effects are observed at different places in the world and at the same place on different occasions due to variation in the filtering action of the atmosphere. For example, the waveband between 290 and 311 nm has been found to constitute roughly 0.08% of sunlight in summer but only 0.008% in winter [92] and explains why a fabric may be yellowed by sunlight in summer but bleached in winter [8].

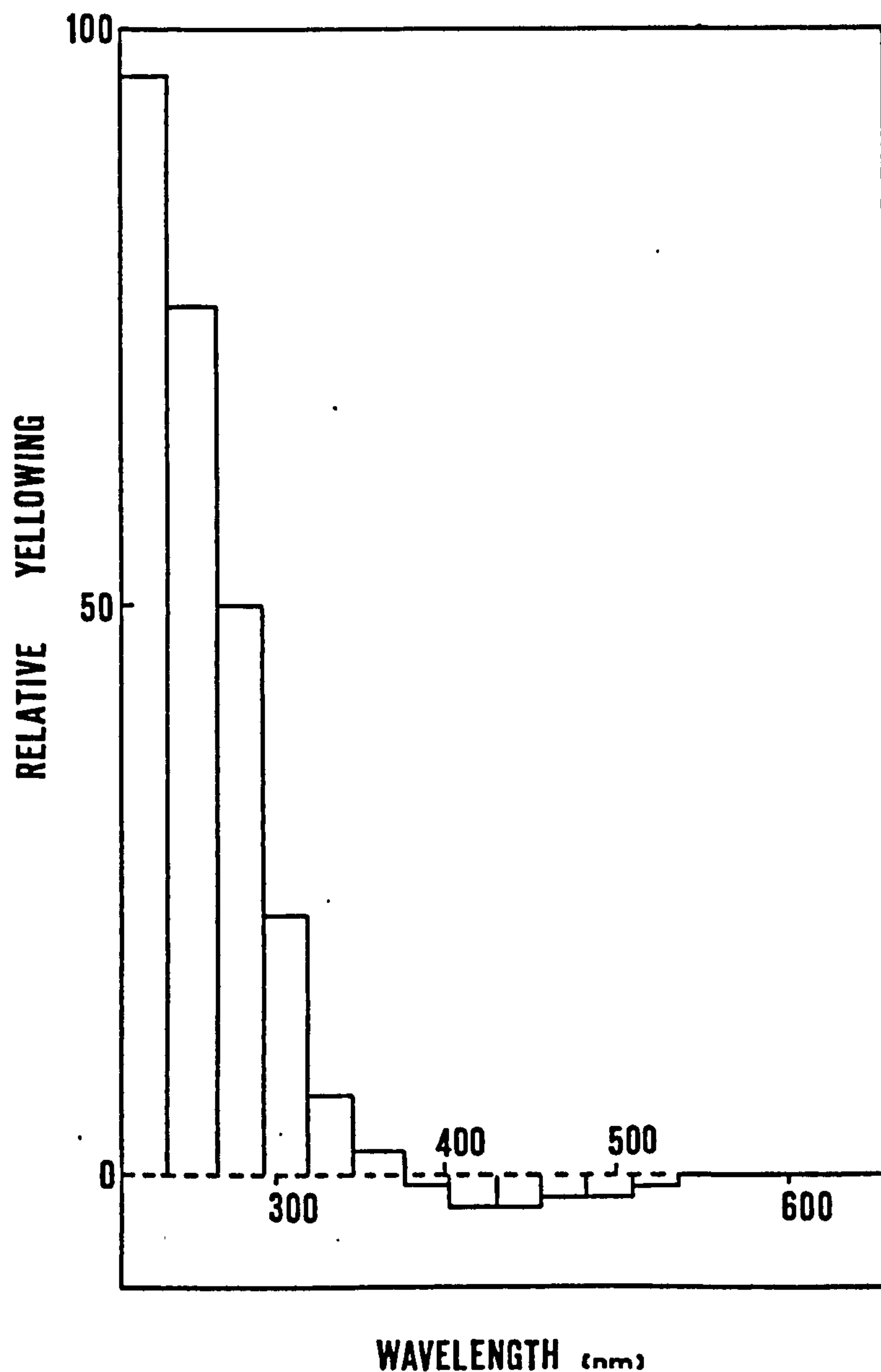


Figure 2.7 Action spectrum for wool (equal energy doses for each wavelength) drawn from results of [91].

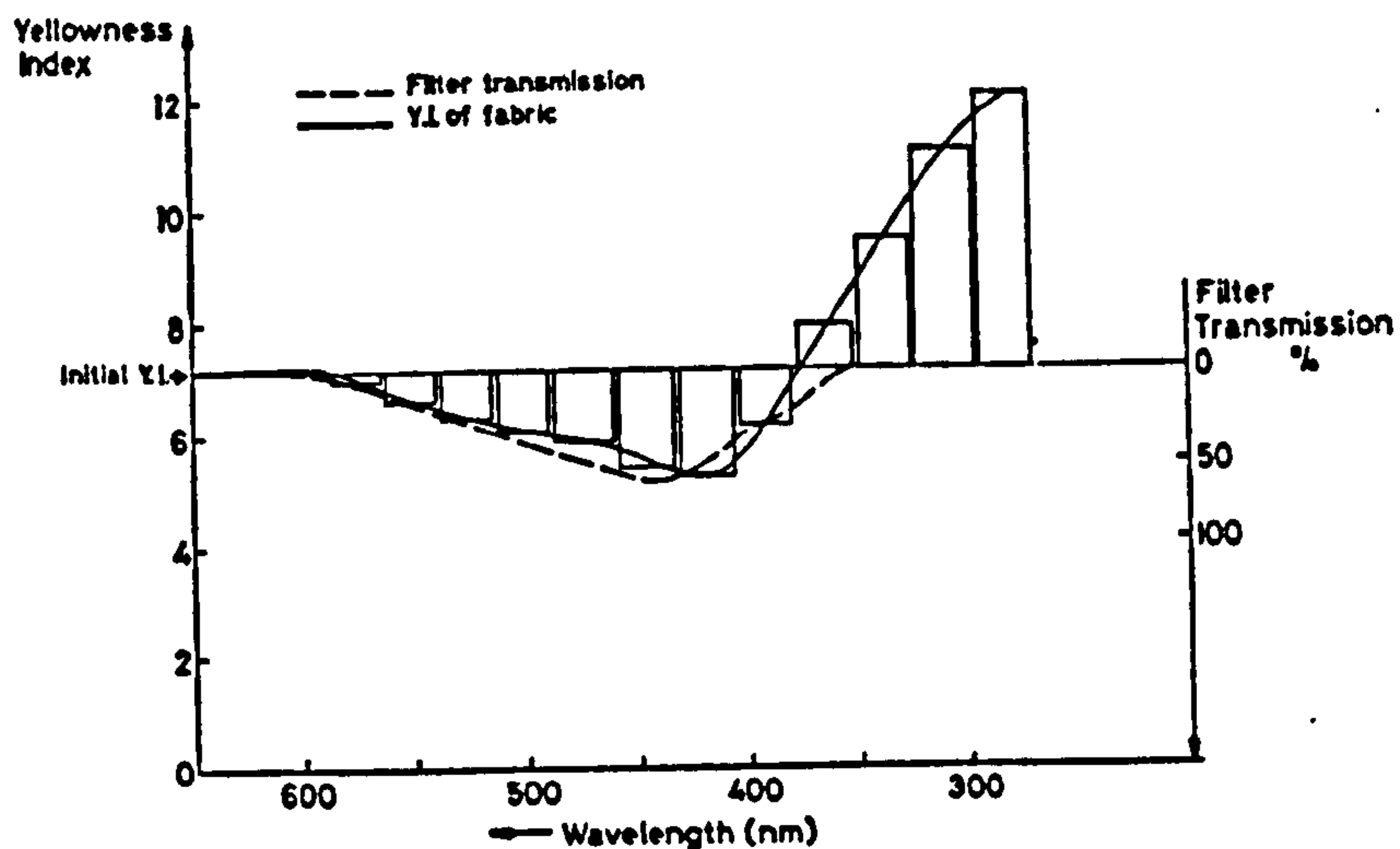


Figure 2.8 Action spectrum for wool obtained for equal energy doses (and % transmission plotted against wavelength for Kodak Wratten filter No. 38A) [89].

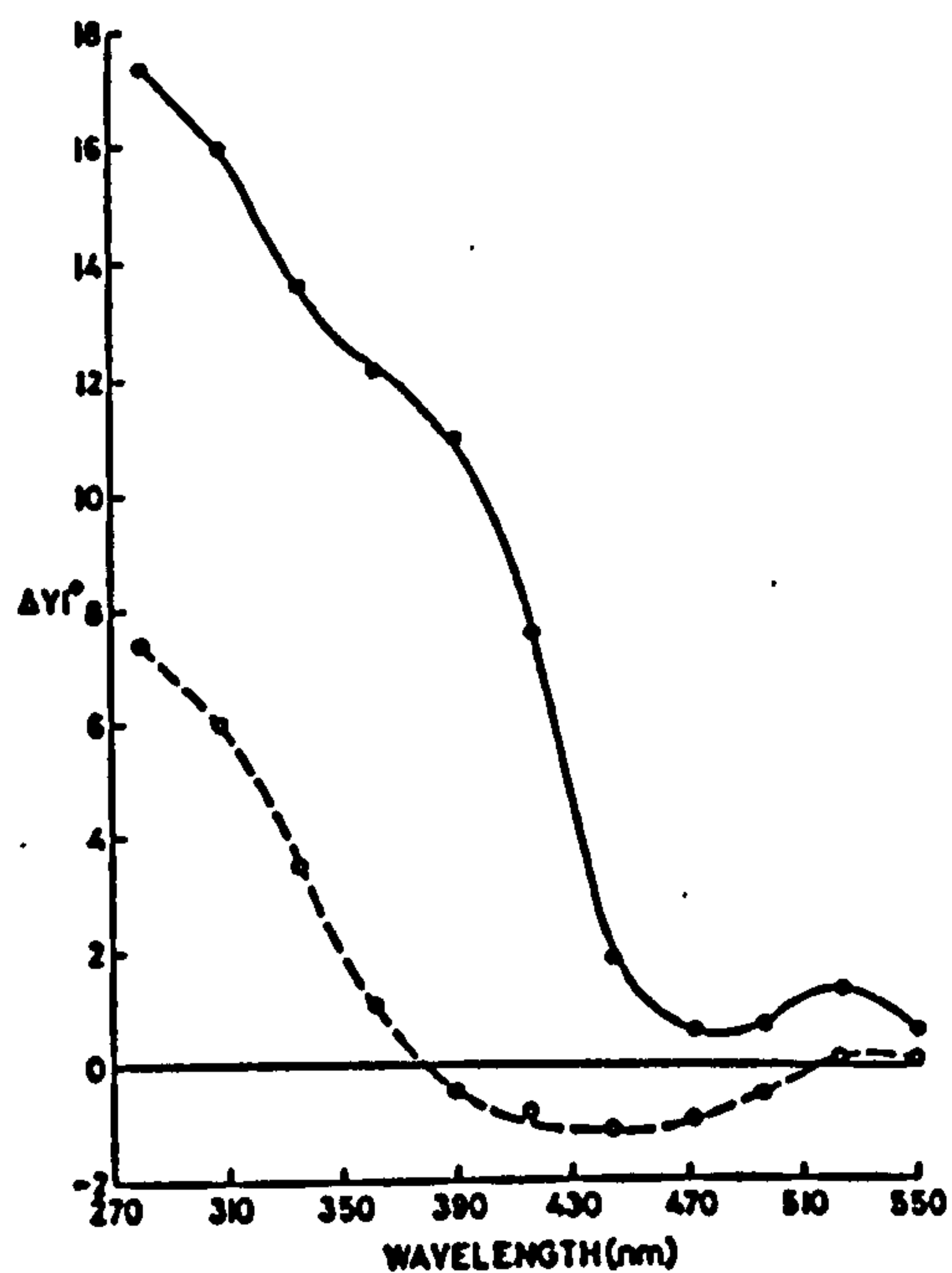


Figure 2.9 Action spectra for yellowing of FWA-treated [—] and untreated [---] wool fabric [90]. The values for $\Delta YI'$ include changes in the fluorescent contribution of the whitener.

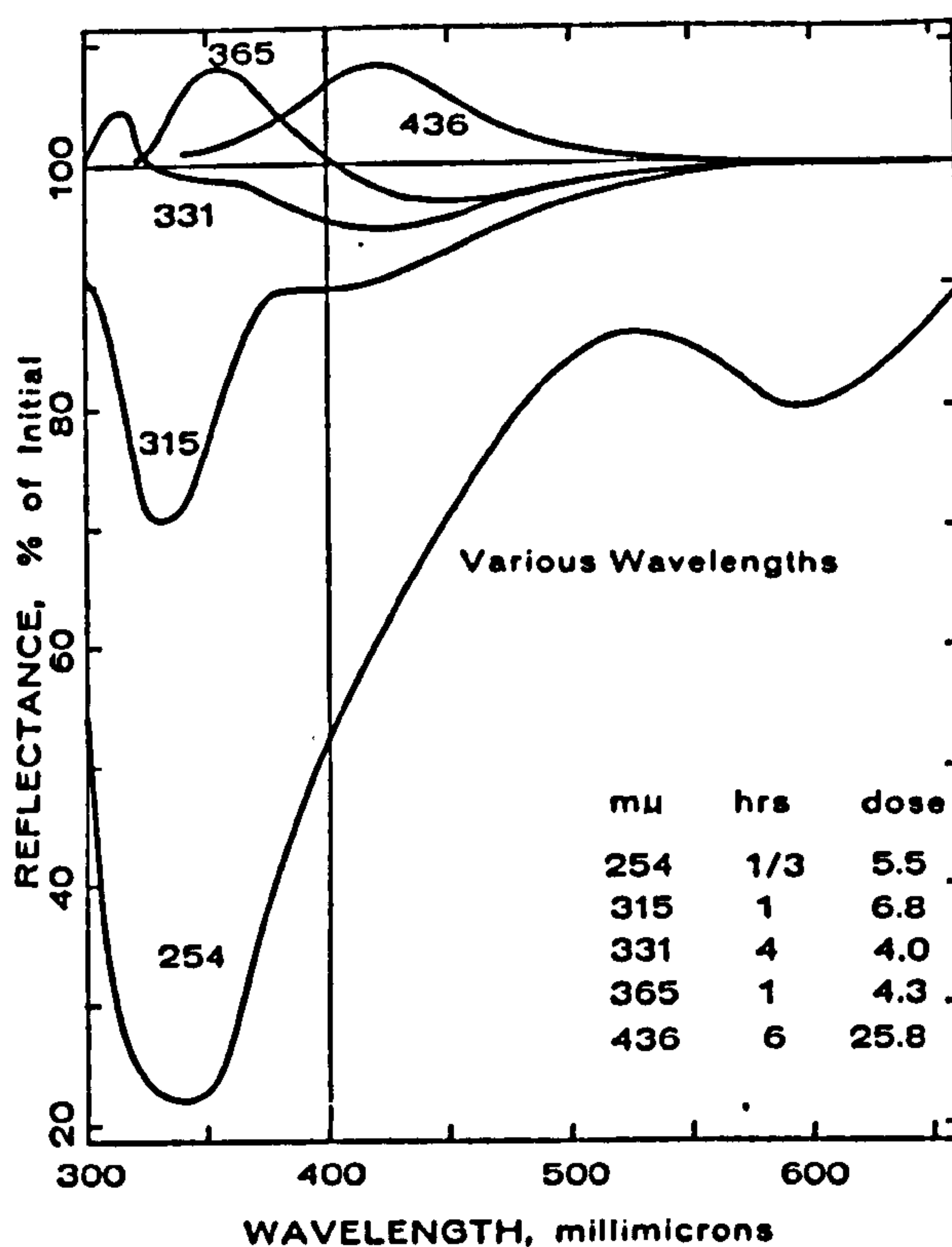


Figure 2.10 Effect on wool of various monochromatic wavelengths [85]. Upper right quadrant: bleaching. Lower right quadrant: yellowing or greening. Left of 400-nm line: invisible increases or decreases of UV reflectance. (Dose is in mW.hr.cm⁻².)

The effect also varies with the initial yellowness of the wool [90]. The more yellow the wool the greater the absorption of blue light and the more rapid the photobleaching action. At the other extreme very white wools undergo only yellowing.

The full effect of exposure to irradiation is not always immediately apparent since irradiation in the dry state may cause further bleaching or yellowing when wool is subsequently wetted [93], and exposure while in the wet state may sensitize wool to additional yellowing if the goods are then heated in water to a temperature which does not affect unexposed wool [94].

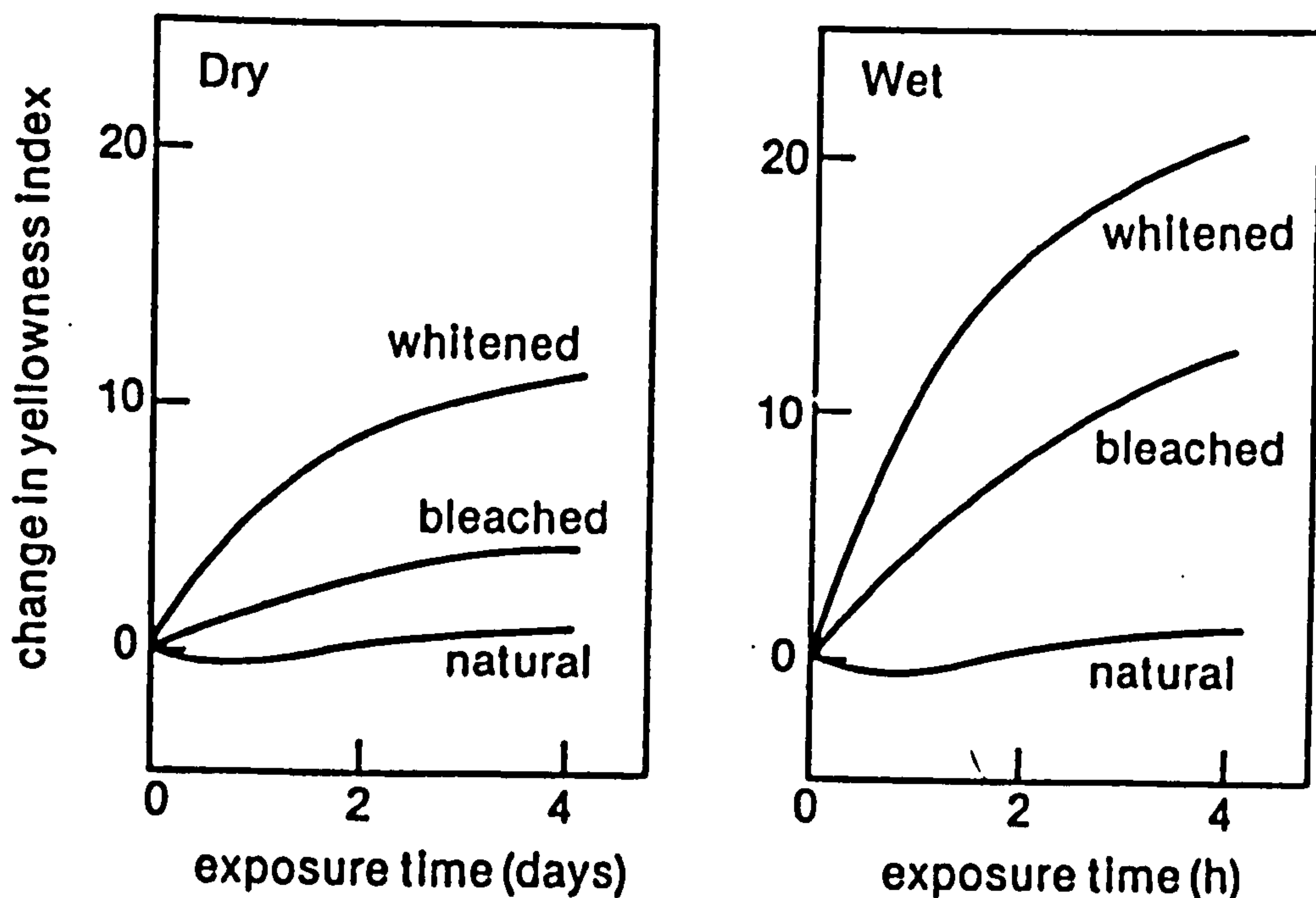


Figure 2.11 Effect of water on the photoyellowing of wool [98]. Rates of photoyellowing of natural, peroxide-bleached, and fluorescently whitened wool fabrics in simulated sunlight.

Both photoyellowing [72,84] and photobleaching [95] are promoted by the presence of water. The rate of photoyellowing is also increased if the wool is chemically bleached [72] or treated with fluorescent whitening agents [96,97] (see Figure 2.11). As wool is generally bleached chemically before the application of fluorescent whitening agents (FWAs) [8], the resultant wool is exceptionally prone to yellowing on exposure, especially if wet.

2.4.3.3 Arising from Weathering

Weathering is the term used to describe the deterioration of wool which occurs as the result of exposure to water and air as well as sunlight during growth [8]. It is more prevalent in open fleeces than in dense ones and is generally confined to the fibre tips, since the root sections are protected from exposure by their coating

of wool grease and suint (see Section 3.2.3.2). The weathered tips are yellow-brown in colour, and much weaker than the unweathered root sections. As a consequence of their brittle nature, most weathered tips are removed in worsted processing.

2.4.4 Photogreening

When irradiated with UV light at 303 nm or shorter (corresponding to 94.4 kcal (395kJ) and above), wool becomes visibly green [69,99,100] owing to formation of chromophores that absorb in the near UV, blue, and orange, leaving a reflectance maximum at 530 nm in the green (see Figure 2.10). The absorbers in the orange, at 600 nm, show a characteristic electron spin resonance (esr) signal due to free radicals [101,102] (Figure 2.12) and are very unstable. The green colour has been attributed to the cystyl radical because the rate of fading of the green colour is much the same as the rate of decay of the broad esr signal attributed to the cystyl radical [103].

The free radicals disappear in the dark in reactions with water vapour and/or oxygen unless kept below -78°C , for example, at which temperature the green colour persists for months or years [104]. At room temperature and above, the orange absorption and its esr signal disappear in minutes, leaving the yellow wool [101].

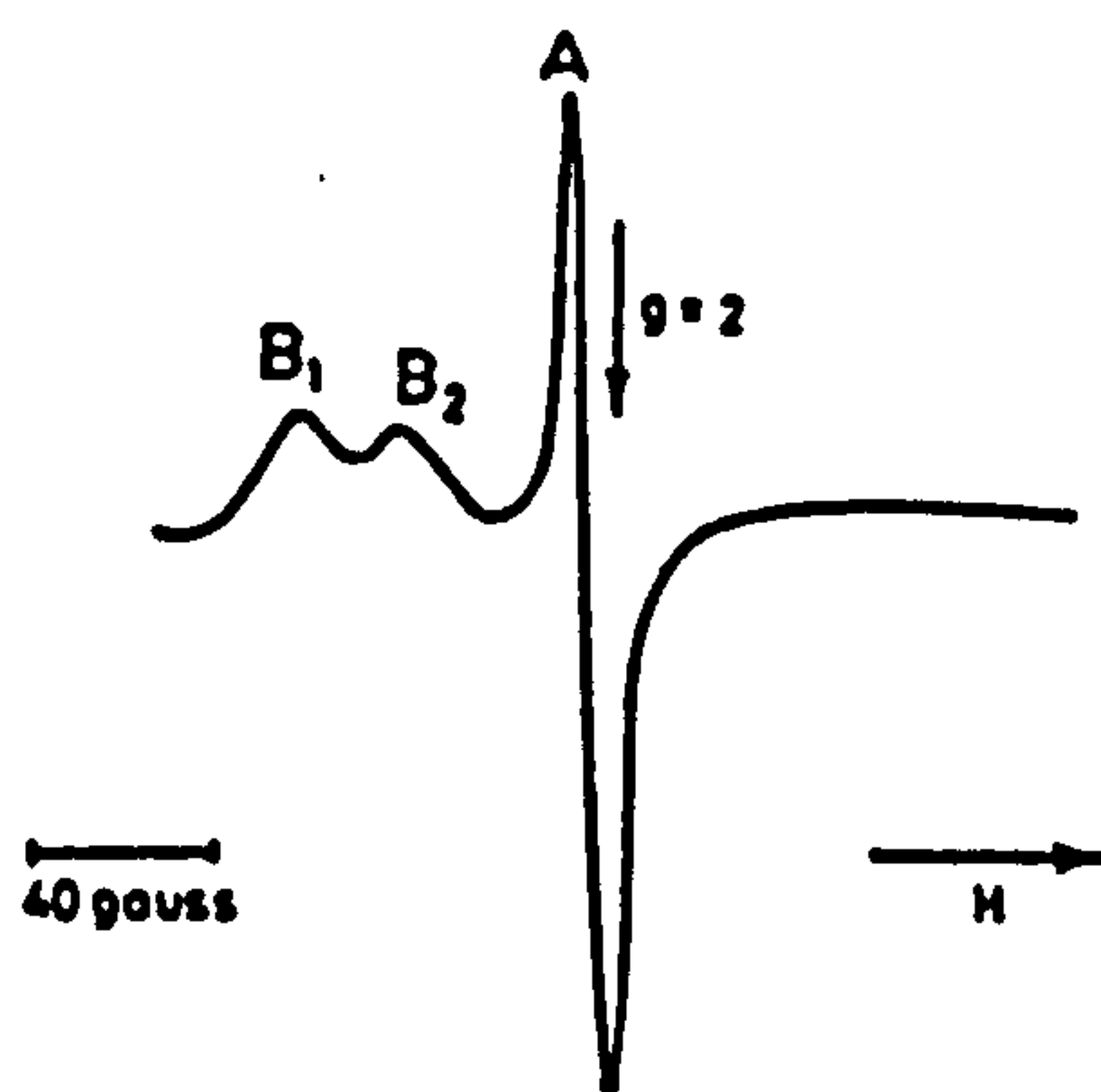


Figure 2.12 Typical esr curve of wool irradiated at 310 nm at room temperature in the absence of oxygen [65].

2.4.5 Photoyellowing

2.4.5.1 Promotion of Photoyellowing

As already mentioned (Sections 2.4.2 and 2.4.3.2) photoyellowing is caused by UV light below 380 nm, increasing with decrease in wavelength, which means that in a typical sunlight spectrum the maximum yellowing occurs in the 340-350 nm region.

However, many factors sensitise wool to photoyellowing. As previously described (Section 2.4.3.2) photoyellowing is promoted by the presence of water or by being chemically bleached or treated with FWAs. It has been shown that wool fails to yellow when irradiated in the absence of oxygen [105]. Indeed a considerable quantity of oxygen is actually consumed during ultraviolet irradiation [62].

Factors in the environment, for example, salts of metals such as aluminium, tin and manganese, also sensitise wool to photoyellowing [90]. It is also known that acid conditions accelerate photoyellowing [106] as does chlorinated solvent retention following a lipid-extraction procedure [107]. Even a mild scour in non-ionic surfactants can cause a significant increase in the rate of yellowing [108].

2.4.5.2 Prevention of Photoyellowing

A number of attempts have been made to devise treatments to protect wool from photoyellowing but these have only been partially successful [65].

1. UV Absorbers. These compounds function by preferentially absorbing the damaging radiation, and then dissipating the energy harmlessly (mainly as heat) [109]. Such compounds are already in commercial use with synthetic fibres with good effect [110,111].

Many UV absorbers have been tested on wool, usually as the sulphonic acid derivative [82,112-116]. Two of the most successful classes are the benzophenones and the benzotriazoles. Generally, however, the protective

effect of UV absorbers is disappointing, although one has recently been commercially launched based on 2-hydroxyphenylbenzotriazole (Cibafast W) which claims markedly improved photostability [117].

UV absorbers are incompatible with fluorescently whitened wool. Preferentially absorbing the same wavelengths as the FWAs their use dramatically reduces the effect of the brightener.

2. Excited State Quenchers. Much evidence has been provided for the theory that wool yellowing occurs as a result of singlet oxygen formation by reaction of triplet excited states with ground state oxygen [118]. It is therefore likely that photoyellowing could be retarded by the effective quenching of these excited states. Most studies have considered only tryptophan, since no other excited states have been identified in untreated wool.

One of the most effective fluorescence quenchers for tryptophan in aqueous solution is acrylamide [119], which is also effective in polymer films [65]. When applied to wool, however, the extent of tryptophan fluorescence quenching was only 25 % of that expected from polymer film measurements and the reduction in photoyellowing of the wool in the presence of the quencher was minimal. To account for this it was suggested that a significant portion of tryptophan residues in wool are located so as to be inaccessible to the quenching molecules applied from aqueous solution.

It therefore seems that this method of stabilisation is not successful with wool, using the quenchers and methods currently available.

3. Antioxidants. These function by destroying primary photoproducts before they can give rise to substrate degradation. Two hindered phenols were found not to offer any protection against photoyellowing of fluorescently whitened wool [43], while recently phosphite esters have been found to improve the light fastness of optically brightened wool [120].

4. Thiourea-Formaldehyde. A process which has been found to be effective is the treatment with thiourea-formaldehyde mixtures [121,122], or precondensates [123], followed by thermal curing. The process minimises the photoyellowing of fluorescently whitened, as well as unwhitened wool, although it is important to whiten the wool before the thiourea-formaldehyde treatment is applied [124]. Although the protective effect is considerably decreased when the treated wool is first rinsed, no further decrease occurs on subsequent laundering [8]. This, together with the treatment being expensive and the chemicals posing a health risk restrict the commercial use of the treatment [118]. The mechanism of protection involves quenching of singlet- and triplet-excited species in the fibre, and sensitised photobleaching by visible light [125].

5. Surface Whitening. Conventional methods of whitening wool distribute the fluorescent whitener more or less uniformly throughout the fibre. Surface whitening is the name coined to describe the application of fluorescent whiteners to the wool surface in a polymer film [126]. This method insulates the whitener from the wool and consequently avoids the sensitisation of yellowing that occurs in conventionally whitened wool.

Many whitener/polymer systems were found to give good whitening [126], though few provide white wool with a satisfactory stability to light, and few are as white as that obtained by conventional methods of application. The photostability of the surface-whitened wool is influenced by the nature of the polymer as well as the FWA [126,127]. The systems with the best light fastness give better stability to light than conventionally whitened wool. The whitening effect is generally found to be stable to washing, but not to dry-cleaning, though stability to dry-cleaning can be achieved by using polymers which can be cross-linked after application, the FWA being bound covalently to the polymer. However, these systems confer an unacceptable harsh handle to the fibre, and whiten wool less effectively than conventional methods.

A variation on this is the research involving the encapsulation of FWAs in cyclodextrins [128] which is currently being investigated.

6. Chemical Modification. Wool has been chemically modified in a variety of ways but with little success. Slight improvements have been observed after treatment with formaldehyde [72], methyl isothiocyanate [129], cyanide [130] or S-methylthioglycollic anhydride [131]. Conversion of the tryptophan residues in wool to oxindolylalanine residues also causes a small decrease in the photoyellowing of wool, especially after prolonged irradiation. A much greater protective effect is observed if the wool is fluorescently whitened after the treatment [132].

7. Reducing Agents. Many investigations, appreciating that the photoyellowing of wool is an oxidation reaction, have attempted to decrease the yellowing of wool and fluorescent whitened wool with reducing agents. Several classes of reducing agents have been examined, which under certain conditions minimise the photoyellowing of wool. These include thiols [133], thiocarboxylic acids [43,133], bisulphite [43, 96, 134], phosphines [43], borohydride [43, 118, 135], hydroxymethane sulphinates [71,118,136] and thiourea dioxide [71,137].

However, the protective effect provided by these reducing agents is largely lost on laundering (where tests have been performed). The protective effect is most evident if the samples are kept wet during exposure and is especially marked if the wool is actually irradiated in a solution of the reducing agent [43,71,134,137]. Both UV [43,71,89,118,134,136,137] and visible light [89,134,138,139] have been used. It has been suggested that in the presence of water the reductants are able to diffuse more rapidly to the photo-sensitive sites in the fibre, where they are able to interrupt the series of reactions which ultimately lead to yellowing [43].

2.4.6 Photobleaching

Irradiation with visible light causes wool to be photobleached i.e. the fabric appears whiter, the effect being at a maximum in the blue region at approximately 450 nm [90]. This bleaching is generally seen as a rapid fading of wool carpets or furnishing fabrics where they have been exposed to sunlight behind glass: in this context, rapid means after several hours. Current fashions favour pastel shades, which can be particularly prone to such photobleaching changes [70].

The terms 'first fade' and 'red-fade' are also used to describe an initial very rapid fading, occurring in less than an hour, of wool exposed to sunlight behind glass [70,79,140,141]. However, these terms are generally used to describe the photobleaching of chlorophyll and its breakdown products that are present in wools that may be stained with dung (pen-stained or dag wools) rather than a photobleaching of the base colour of clean wool [79,140,142].

A number of factors affect photobleaching. The greater the initial yellowness of the wool (however caused) the greater the photobleaching [88,141-143]. Photobleaching is increased by moisture [142,144], decreasing pH [144], chemical prebleaching [142,145] as well as phosphate and aluminium ions [144]. Common organic solvents [144] have no effect while there is a minor temperature dependence [142]. Exclusion of oxygen prevents photobleaching [66].

Impregnation of wool with reagents such as sodium hydrosulphite, thiourea dioxide, thioglycolic acid and thiourea-formaldehyde resins accelerate photobleaching [138] (see Section 2.4.5.2). The use of a zinc complexed thioglycolic acid has been proposed both as a photobleaching catalyst, and as an additive for detergents [89].

The photobleaching of wool has been advocated as an alternative to chemical bleaching [95,146]. Such problems as shrinking and felting are eliminated, as is the decrease in tensile strength [146]. The process is not used commercially as the cost is prohibitive [95]. It is a common observation that prolonged exposure of human hair to sunlight results in hair bleaching [147] and a commercial process for photobleaching hair has recently been proposed [147].

Wool that has been photobleached artificially is at least as [146], if not considerably more [142], photostable on subsequent sunlight exposure. Whilst it has been found that compared to chemically bleached fabric photobleached fabric has a less stable colour, particularly if the bleached wool is subsequently boiled in a conventional dyeing process [139], it has also been found that wool photobleached using a zinc thioglycolic acid catalyst gives a better light-fastness than peroxide bleached wool [89].

Using blue light it was found possible to bleach only half of the initial yellow colour of natural and artificially yellowed wool [88]. From this it was suggested that two chromophoric groups may be present, only one of which is susceptible to blue-light bleaching.

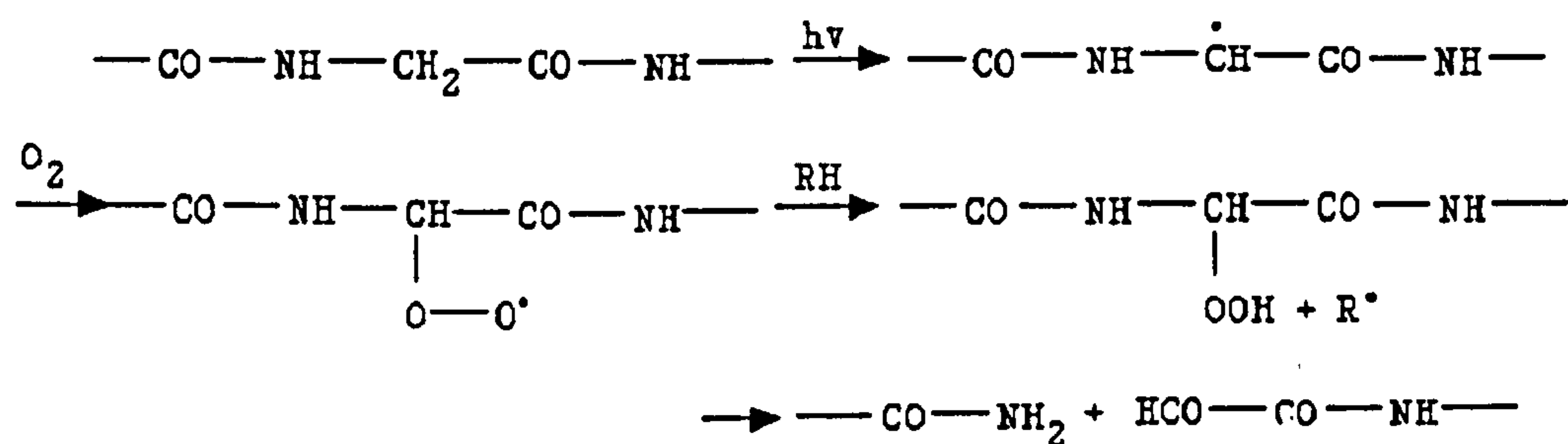
Neither the chromophores responsible for the initial colour of natural wool nor those produced by irradiation are known [8]. Irradiation of wool with blue light ($\lambda=400-450$ nm) is claimed to produce two predominant species of free radical as well as at least two other minor species [148,149]. Of the predominant species one is believed to originate by hydrogen abstraction at α -carbon atoms, and the other by decarboxylation [149,150]. One of the minor signals is usually assumed to be associated with sulphur radicals but either fails to appear or appears only after prolonged illumination, subsequent to the appearance of other signals [150].

The photobleaching of wool with blue light is the most common example of wool undergoing 'spectral conformity' [86,151] which is where exposure to light causes reflectance and transmittance to rise near the wavelength of the light because the photons eliminate their particular absorbers, leaving the remainder of the chromophores unchanged, rather than cause new absorbers. Thus, wool for example, becomes bluer in blue light, greener in green light and whiter in white light. Spectral conformity seems to be a general phenomenon as many other substances undergo it [151].

2.4.7 Photochemical Reactions

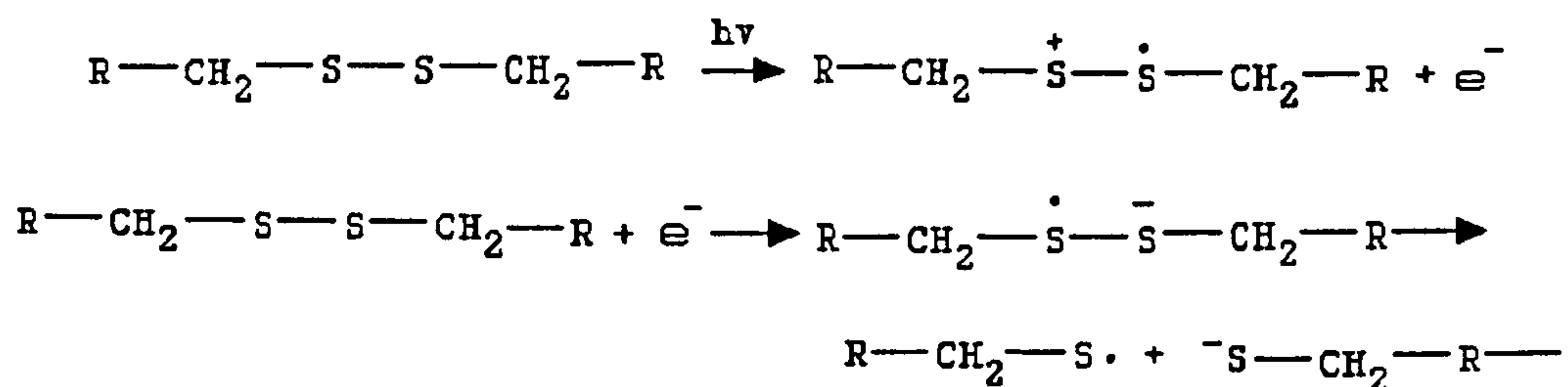
The photochemical reactions of wool have been reviewed [8,65,66,109].

Exposure of wool to sunlight causes both main chain and side chain photo-oxidation [65]. Amino acid analysis of the hydrolysates of irradiated wool has revealed that tryptophan, histidine and cystine suffered the most degradation with tyrosine, phenylalanine and methionine also suffering significant losses [152]. The identification of small amounts of glyoxylic and pyruvic acids in the protein hydrolysate indicates breakdown at the alanine and glycine residues according to the following mechanism [153]:



The subsequent identification of a number of other α -keto acids in the hydrolysates of irradiated wool [154] suggests that main chain breakdown is not confined to the alanine and glycine residues but can occur adjacent to many other amino acid residues [65].

The reactions proposed above lead to the formation of free radicals. The presence of free radicals in UV irradiated wool can be readily observed by esr spectroscopy but due to a lack of distinct hyperfine structure of the resulting spectra they are sometimes difficult to identify. A typical esr spectrum obtained from wool following irradiation at room temperature with a narrow band of energy at 310 nm is shown in Figure 2.12 and comprises essentially an intense singlet line (A) at $g \approx 2.00$ and an asymmetric low field signal (B_1 and B_2). This latter signal is readily identified with the thiyl radical $\text{R-CH}_2\text{S}^\bullet$, formed either by the homolytic fission of the disulphide bond of cystine or by the rearrangement of a cystinyl radical anion (formed by trapping an electron) [155], for example:



This disulphide bond breakdown, together with the main chain breakdown, is chiefly responsible for the phototendering of wool.

The singlet line spectrum (A) was originally designated as a combination of an alanyl radical $-\text{NH}-\dot{\text{C}}(\text{CH}_3)-\text{CO}-$ and a glycy radical $-\text{NH}-\dot{\text{C}}\text{H}-\text{CO}-$ [156], which agrees with the chemical evidence following the isolation of glyoxylic and pyruvic acids [153]. However, this designation was queried [157] after it was found that the singlet line spectrum can be resolved into a doublet and a quartet [157]. It has been proposed that the doublet was due to an unpaired electron associated with the peptide carbonyl oxygen, $\text{>C=O}\cdot$, and the quartet with an unpaired electron located on the γ -carbon of proline [65]. At present there is no chemical evidence to support this designation.

These designations of free radicals in wool which has been UV irradiated do not include radicals associated with the strongly absorbing and photolabile tryptophan residues [65], despite the fact that a free radical, readily observed by esr is formed by the irradiation of aqueous solutions of tryptophan [158]. This species has been identified as the 3-indolyl radical, formed by the rearrangement of the 1-indolyl radical following N-H bond fission [158] and/or photoejection of an electron with subsequent deprotonation [159]. However, no such radicals could be observed when tryptophan was irradiated in poly(vinylalcohol) (PVA) films [160]. It has been shown [161] that during the UV irradiation of tryptophan in aqueous solution both photoejection of electrons and N-H bond fission contribute to radical formation at wavelengths less than 275 nm (which equates to the photoionisation energy threshold for tryptophan in water) but at longer wavelengths only N-H bond fission contributes. For tryptophan in isopropanol the photoionisation energy threshold is less than 250 nm [161], a value which might be expected to apply to

PVA films, hence no photoejection of electrons would be expected from tryptophan in this polymer for radiation with wavelengths higher than 250 nm. Following UV irradiation some tryptophan radical formation by N-H bond fission would be expected to occur in the polymer film but apparently not to a sufficient extent to form radicals in detectable quantities. Consequently radical pathways would appear to play a very minor role in the photodegradation of tryptophan in PVA films with radiation of wavelengths in excess of 300 nm, and by deduction in wool under the same irradiation conditions [65].

When wool is irradiated with wavelengths higher than 360 nm, an increase in the naturally occurring esr signal occurs [162]. This signal, which is stable to air and has a quite different esr spectrum to that attributed to radicals produced by radiation of wavelengths lower than 325 nm, has yet to be identified [65]. Since this spectrum is very similar in stability and shape to that obtained from pigmented wool [64], it has been suggested that the signal may be formed from some pigment precursor in the unpigmented wool. It may be due to the same unidentified species which has been detected in the UV absorption and phosphorescence spectra of wool [62]. An alternative proposal [163] is that this stable radical is due to the presence of a ferric ion-keratin complex, resulting from absorbed iron in the wool.

A large amount of research has been carried out to explain the photochemical reactions leading specifically to wool yellowing and a number of theories have consequently been produced. It has been proposed [153] that wool yellowing was due to the visible light absorbed by the α -keto acids formed by the photodecomposition of the polypeptide chain. However, it has been shown [154] that whereas the irradiation of dry wool causes an increase in carbonyl groups, their rate of production is approximately the same for unbleached, bleached and fluorescent whitened fabrics, yet these fabrics yellow at noticeably different rates. Additionally, irradiation of the same fabrics in water resulted in only a slight increase in carbonyl group formation although extensive yellowing occurred. It was concluded that the formation of carbonyl groups was unlikely to be responsible for wool yellowing.

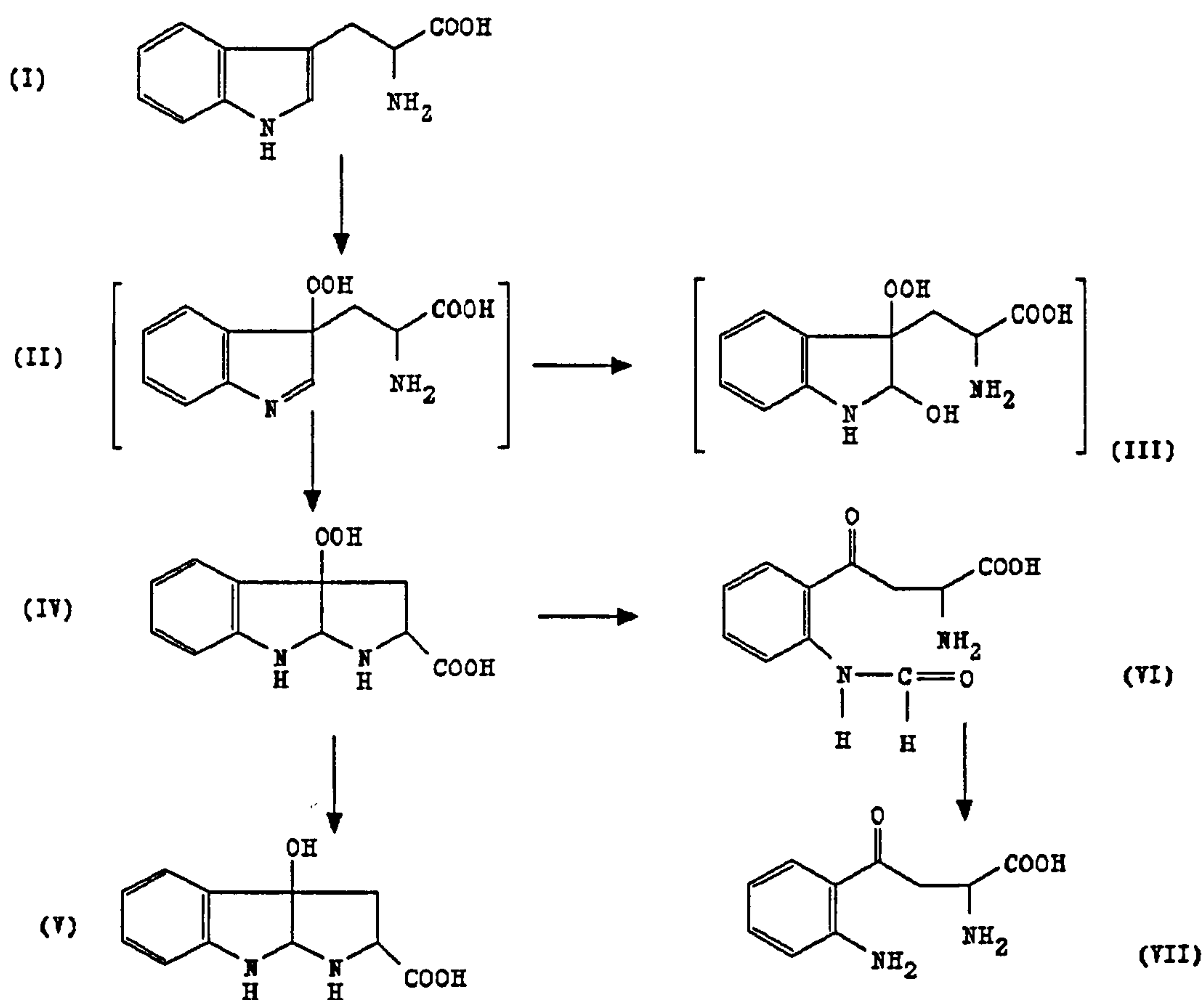
Another proposal [164] is that the inherent yellowness of wool was due to the presence of mobile electrons which could be readily photo-excited into conduction bands. Extra yellowing by alkali, heat and UV irradiation was due to an increase in the number of mobile electrons following the introduction of additional unsaturated groups into the fibre. The fact that in irradiated wool esr studies could find no evidence for electrons in conduction bands [162] and that yellow compounds can be isolated from photoyellowed wool by enzymatic digestion [165] cast doubt on this proposal.

The most widely held theory of wool yellowing proposes the photodecomposition of the tryptophan residues to form yellow compounds. Considerable evidence is now available to support this proposal [65].

Tryptophan is the amino acid most susceptible to sunlight yellowing both in polymer films [160] and in aqueous solution [166]. A strong correlation was observed for the rates of yellowing of 29 different keratin samples with their initial concentration of tryptothan and with the destruction of tryptophan [108]. Correlations of yellowing with the destruction of other amino acids were less significant. A significant increase in yellowing was achieved by the incorporation of tryptophan derivatives into wool [167]. Some yellow products of photodegraded wool were isolated by enzymatic digestion [165] but only one product, kynurenine, a recognised oxidation product of tryptophan, could be identified. Additionally when the tryptophan residues in wool were radioactively labelled, the yellow photoproducts were likewise radioactive, thus directly implicating tryptophan as a major source of yellowing.

To avoid the problems associated with the isolation of peptide-bound photoproducts from an insoluble substrate such as wool, the photodegradation in aqueous solution of free tryptophan and its derivatives has been widely investigated [65]. Two types of studies have been performed, the first involving direct photolysis, and the second using a photosensitising dye. Most sensitising dyes used have been singlet oxygen generators [168] and the photo-oxidation of tryptophan and its derivatives was the result of the reactions with singlet oxygen.

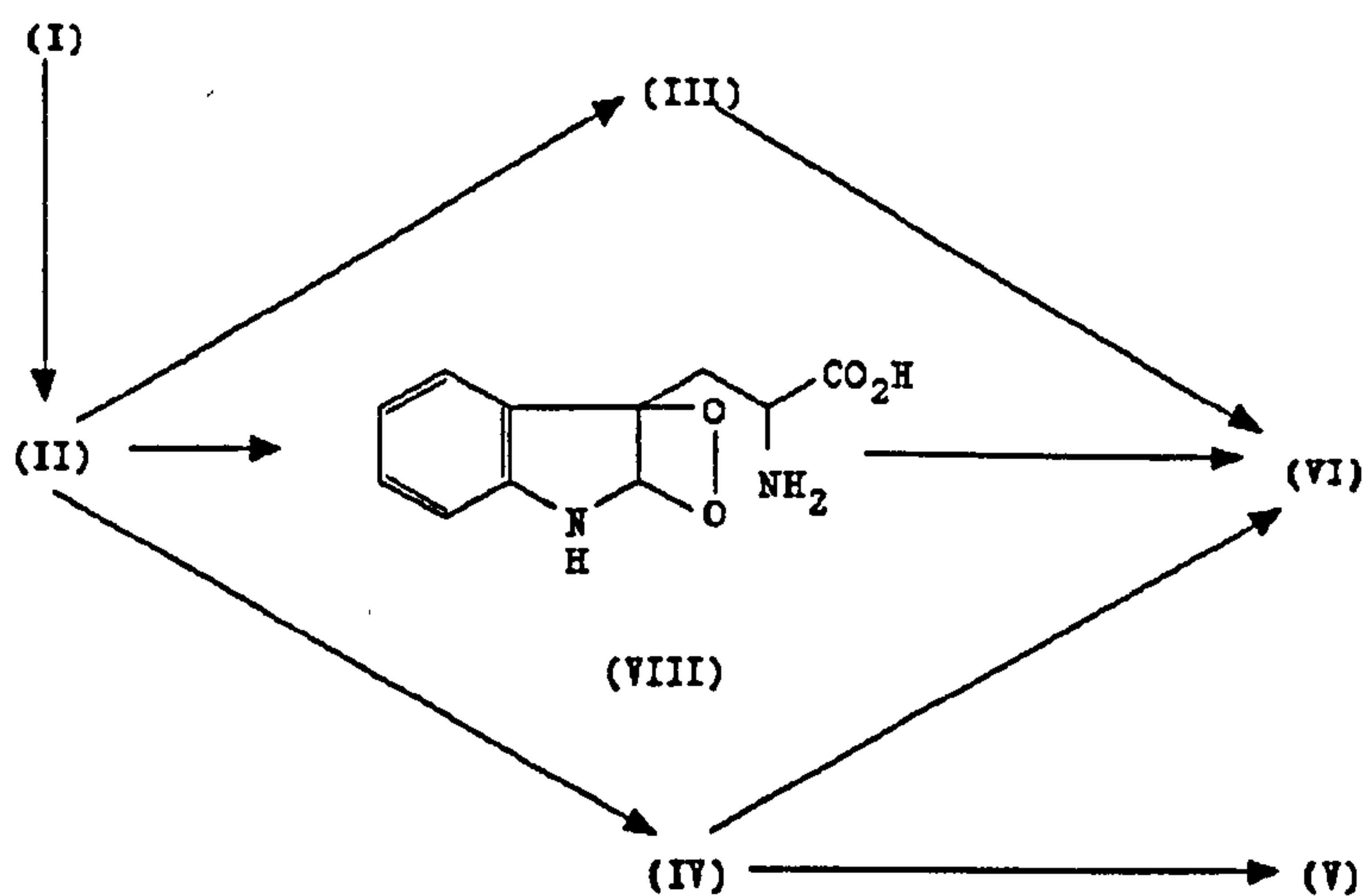
The complexity of the reactions involved in the direct photolysis of tryptophan is illustrated by the finding of at least nine degradation products after the exposure of an aerated aqueous solution of tryptophan to a flash photolysis lamp [169]. The short duration of the flash (15 μ s) ensured that most of the products, which have not yet been identified, were essentially primary reaction products. Following prolonged irradiation of tryptophan (I), N-formylkynurenine (NFK, VI) [170-171], kynurenine (VII) [172] (both intensely yellow coloured compounds, which are fluorescent [90]), 3a-hydroperoxypyrrolidinoindole (PPI, IV) [173] and 3a-hydroxypyrrolidinoindole (HPI, V) [173] have been isolated and identified. It has been proposed that the short-lived intermediate II (indolenine hydroperoxide) is involved in the formation of products IV and V according to Scheme 2.1.



Scheme 2.1 [173]

Such a reaction scheme would be applicable to the situation where the α -amino group of tryptophan is free, allowing the lone pair on the nitrogen to attack the indole in position 2 [65]. However when tryptophan is incorporated into a protein chain the ring closure in transforming II to IV would be restricted by both the loss of activity of the nitrogen bound into an amide group and by steric factors. This loss of activity probably accounts for the lower rate of yellowing of tryptophan in aqueous solution following acetylation of the free amino group [174].

As singlet oxygen is involved in both the direct photoyellowing of wool (see later) and the fluorescent whitener photosensitised yellowing of wool [65], then the dye-sensitised photo-oxidation studies of tryptophan, where the major reactant is singlet oxygen, become very relevant. The products obtained by dye-sensitised photo-oxidation of tryptophan have included NFK (VI) [175], PPI (IV) [176], and HPI (V) [177,178]. It has been proposed [177,178] that an indolenine hydroperoxide (II) was the initial product which converts to either NFK or HPI by alternative pathways. Subsequently PPI has been isolated as a major product [176] and it was found that either it reduces to HPI or readily rearranges to NFK, leading to reaction Scheme 2.2.



Scheme 2.2 [176]

This reaction is very similar to that proposed earlier for direct photolysis (Scheme 2.1) and the restrictions noted for protein-bound tryptophan in that instance would also apply here. The formation of PPI (IV) and ultimately HPI (V) from protein-

bound tryptophan in wool must consequently be considered as highly unlikely [65]. However, the intensely yellow coloured NFK (VI) would be a photoproduct, formed, not via intermediate PPI, but by other routes, for example via the energetically unfavourable dioxetane (VIII) [176]. The fact that kynurenine (VII), formed by the removal of the labile formyl group from NFK, has been isolated from photoyellowed wool [165] shows that such pathways occur.

While these findings clearly implicate tryptophan residues in the photochemical yellowing of wool, they give very little indication of the primary photochemical reactions involved, apart from the fact that free-radical reactions are not involved to any extent [65].

Wool absorbs energy at wavelengths less than 320 nm due to the amino acids tryptophan and tyrosine [65]; however, the energy absorbed by tyrosine is efficiently transferred to tryptophan, so that all the energy absorbed initially resides in the singlet excited state of tryptophan. Only about 8% of this energy is lost by fluorescence [179]; the remainder is dissipated by internal conversion, quenching within the protein, e.g. by disulphides [180,181] and by intersystem crossing to the triplet state. A phosphorescence quantum yield of 0.13 for the tryptophan emission peak of wool at 77K indicates that considerable intersystem crossing to the tryptophan triplet state occurs [62].

For wavelengths higher than 320 nm, energy absorption is due to an absorbing species which is as yet unidentified [65]. Its singlet excited state apparently undergoes similar deactivation pathways to that of the tryptophan singlet state, including intersystem crossing to the triplet state, which at 77K has a phosphorescence quantum yield of 0.06 [62]. Hence, two distinct triplet state molecules are formed concurrently when wool is exposed to sunlight. In both cases their rates of decay are of first order in the presence of oxygen and are accelerated by the presence of moisture [62]. It has been observed that for every molecule of tryptophan destroyed at least ten molecules of oxygen are consumed, regardless of whether the wool is irradiated with wavelengths above 340 nm or below 320 nm [62].

To explain these results it has been proposed [62] that the triplet excited states of the unidentified species absorbing at 340 nm and of tryptophan react with ground state oxygen to produce singlet excited oxygen by energy transfer:



The singlet excited oxygen then diffuses through the wool keratin to react with the amino acids methionine, histidine and tryptophan - the three amino acids known to react with singlet oxygen [182] - and possibly with the unidentified absorbing species. The rate of diffusing of both ground state and singlet excited state oxygen is enhanced due to the fibre swelling caused by the presence of moisture [65].

To corroborate this proposal it would be necessary to show the formation of singlet oxygen in irradiated wool and that wool reacts with singlet oxygen to produce yellow discoloration, both of which are experimentally difficult [65]. However, it has been observed that when sodium azide, a known singlet oxygen quencher, is applied to wool, the rate of oxygen consumption and the rate of tryptophan degradation are both reduced [62]. Additionally by passing oxygen over irradiated wool fabric, $6\mu\text{Mg}^{-1}\text{h}^{-1}$ of singlet oxygen was detected [62]. Very recently [276] direct evidence for the involvement of singlet oxygen has been provided by the observation of the weak phosphorescence emitted by the $^1\Delta_g$ state of singlet oxygen upon UV irradiation of wool.

Additional indirect evidence comes from the fact that wool that has been chemically bleached, e.g. by alkaline peroxide, is much more susceptible to photoyellowing than unbleached wool [72] and also has a much increased phosphorescence [183]. Hence, this greater phosphorescence, resulting in an increased yield of singlet oxygen, could account for the increased yellowing. The larger phosphorescence in bleached wool has yet to be identified [65], but could be due to the oxidation of certain amino acids, especially tryptophan, or of other substances in the wool, to more phosphorescent products. Alternatively, the cystine quenching of the excited states in wool would be decreased by the oxidation of cystine, thus enhancing the normal phosphorescence.

Many reports dealing with the sunlight yellowing of wool make such comments as 'most of the light absorbed by wool is due to tryptophan residues' and others that 'the most damaging wavelengths are those in the 290-310 nm wavelength region of the spectrum' [65]. However, if the action spectrum of wool yellowing (Figures 2.7-2.9) is corrected for the energy distribution in sunlight, the maximum yellowing is found to occur in the 340-350 nm region, which is where the unidentified absorbing species has its absorption peak [65]. Consequently, it would seem that when wool is exposed to sunlight, as opposed to artificial sources, yellowing is more dependent on the absorption by the 340 nm absorbing species than on that of its amino acid components. Regardless of which species is the major source of phosphorescence, oxidation of tryptophan by singlet oxygen is the probable cause of subsequent yellowing [65]. Interestingly, it has been found that the excitation spectrum of the delayed fluorescence of Rhodamine B on wool does not correspond to the tryptophan absorption curve but to the absorption curve of the unknown phosphorescent substance which absorbs maximally at 340 nm wavelength [184]. Hence, identification of this absorbing species, the behaviour of its excited states and its reactivity with singlet oxygen would help to understand better the reactions leading to wool yellowing [65].

While there is much evidence to support the theory that singlet oxygen is involved in the yellowing of wool it must be stressed that photochemical reactions are invariably quite complex and that other reactions may also be contributing to the discoloration [65].

2.4.8 Weathering

Most of the research reported so far has used artificial light or filtered sunlight. In nature the effect of all the sunlight spectrum as well as water and air over a long period of time is experienced. This effect - weathering - is best seen in the tips of fibres from dense fleeces. The change in colour that arises has already been described (Section 2.4.3.3).

Weathered wool swells much more than unweathered wool when immersed in dilute alkali [185,186]. This is a consequence of the photo-oxidation of cystine residues to cysteic acid residues (see Table 2.6) [187-190], which not only cleaves the disulphide crosslinks that restrict swelling, but also introduces charged sulphonate groups that promote swelling by mutual repulsion.

The difference in swelling behaviour can cause ‘tippy’ dyeing, a phenomenon in which weathered fibres often accept more dye than unweathered fibres (although depending on the dye used the reverse can be true) [61,191,192]. Many dyes diffuse more readily into weathered tips than into the root sections, and thus preferentially dye the weathered tips. The increased rate of diffusion of dyes into weathered wool may also be partly due to damage to the epicuticle [188]. (The epicuticle normally retards the diffusion of dyes into wool fibres [8].)

Amino Acid	Composition (mol. 100 mol ⁻¹)	
	Tips	Roots
CySO ₃ H	0.402	0.207
Asp	6.758	6.723
Thr	6.853	6.742
Ser	10.641	11.033
Glu	13.102	12.597
Pro	5.485	6.179
Gly	8.473	9.222
Ala	6.051	5.695
Val	5.680	5.422
Cys	6.484	6.210
Met	0.466	0.477
Ile	3.153	3.073
Leu	8.369	8.141
Tyr	3.937	4.393
Phe	2.786	2.836
Lys	3.050	3.072
His	0.820	0.900
Arg	7.492	7.108

Table 2.6 Amino acid composition of Merino tips and roots [190]

2.5 LUMINESCENCE OF WOOL

2.5.1 Early Studies of the luminescence of proteins and wool

Becchari in the eighteenth century was probably the first to encounter the phosphorescence of proteins when he found that ‘... if a person shut up in a dark room puts one of his hands out into the sun’s light for a short time and then retracts it, he will be able to see the hand distinctly and not the other’ [193]. Stokes observed the fluorescence of horns and nails (cited by Dhéré [194,195]).

Regnaud in 1858 [196] was the first to describe the fluorescence of the crystalline lens. Sechenov in 1859 [197] observed the fluorescence of skin, and in 1883 Soret [197] described the violet fluorescence of myosin in 1% hydrochloric acid. Helmholtz in 1896 [198] observed the fluorescence of vertebrate eyes, and Hess in 1911 [199,200], that of insect and crustacean eyes. Stübel in 1911 [210], using ultraviolet light was able to observe a bluish white fluorescence from various tissues. Nails and unpigmented hair were found to fluoresce more strongly than skin.

Hirst in 1927 [202] while using UV light for examining textiles appears to be the first to report the fluorescence of wool and also, the effect of light exposure on the fluorescence of wool. He found that ordinary normally scoured wool gave a bright blue fluorescence while wool purified by alcohol extraction had a very white fluorescence. He also found that exposure to sunlight destroyed the fluorescence of wool yet at the same time observed that Australian Merino greasy wool exhibited a fluorescence which was yellow in colour with bluish-white tips! Wool dissolved in caustic soda gave a solution which was strongly fluorescent and similar to wool itself. Precipitation of the dissolved wool by acidifying the solution produced an amorphous precipitate which was also fluorescent, while the filtrate, even in considerable dilution, also had the same fluorescent colour as wool.

Sommer in 1928 [203] using UV light found that both alkali (corresponding to 30% loss in strength) and acid damaged wool were indistinguishable from undamaged wool. However, Ellinger in 1930 [204] reviewing the use of UV rays in textile investigations says that the general phenomenon of textiles fluorescing has 'been known a long time, and gave rise to the fluorescence microscope of Lehmann, which is manufactured and sold by Zeiss, of Jena'. Ellinger also reports previous studies by Nopitsch on the fluorescence of textiles. The results concerning wool in descending order of brightness, were:

- '(1) Bleached wool (Zephyr yarn) shows quite the strongest fluorescence; irradiates a clear, bluish-white light. Colour in daylight, white.
- (3) Clear-coloured Unbleached Wool (Tops) emits a clear blue light ...; colour in daylight, pale gold.
- (7) Carding Wool (White Raw No. 15) emits a bluish light, with a clear tendency towards yellow, the colour of the light at times hidden in the yellow; colour in daylight, brownish gold'.

Henk in 1937 [205], Loreti also in 1937 [206], Dervaux in 1939 [207] and Reeder and Nelson in 1940 [208] all report wool fluorescing. Loreti found that wool gave an intense violet fluorescence using UV light and that its intensity was little changed by the addition of 0.1% quinine sulphate in the absence and presence of 10% formaldehyde.

Millson in 1943 [209] appears to be the first to report the phosphorescence of wool and also, the effect of light exposure on the phosphorescence of wool. He found that exposure of wool for 64 hours in a Fadeometer caused the fabric to become whiter having a duller and more violet fluorescence (UV excitation) with about the same rate of decay in phosphorescence although the colour of the phosphorescence was yellower. (The 'Fadeometer' glass-enclosed carbon arc radiates mainly in two broad cyanogen bands peaking at 389 and 359 nm, which extend continuously from 290 to 450 nm, above which about half as much energy is also radiated in a broad white continuum [143]).

Millson also examined the phosphorescence and fluorescence of various undyed yarns. The results for wool are reproduced in Table 2.7. He found that samples which were bone dry exhibited greater phosphorescence, while damp or wet

samples did not phosphoresce at all. The duration of phosphorescence of wool was found to increase from 10 seconds to 17 seconds as the length of exposure was increased from 15 seconds to 3 minutes (1 minute exposure was used for the yarns in Table 2.7).

Wool Yarn	Fluorescence	Colour of Phosphorescence	Duration of Phosphorescence
Wool	Yellowish White	Colourless	12s
Wool, peroxide bleached	Bright Bluish White	Yellowish White	13½s
Wool, treated with soluble sulphoxalate	Bluish White	Yellowish White	18½s

Table 2.7 Phosphorescence and fluorescence of undyed wool yarns (from [209]).

2.5.2 Fluorescence of Wool

2.5.2.1 Spectral Characterisation

The fluorescence emission is dependent on the excitation wavelength which indicates that more than one species is responsible for the emission. Emission spectra have been obtained over the excitation range of 265 to 445 nm [90,118,179,180,184,190,210-220], resulting in emission maxima of approximately 340 nm for 265 nm excitation [210], and 510 nm for 445 nm excitation [215] respectively. A three-dimensional map as well as a contour map in excitation - emission wavelength space can be obtained by systematic scanning of fluorescence excitation and emission wavelengths (Figures 2.13 and 2.14). Clearly visible are maxima in excitation at 285 nm and 335 nm with corresponding emission maxima at 340 nm and 420 nm respectively. Also apparent is a ridge extending to longer excitation and emission wavelengths with a shoulder at an excitation wavelength of approximately 380 nm and a corresponding emission wavelength of approximately 460 nm.

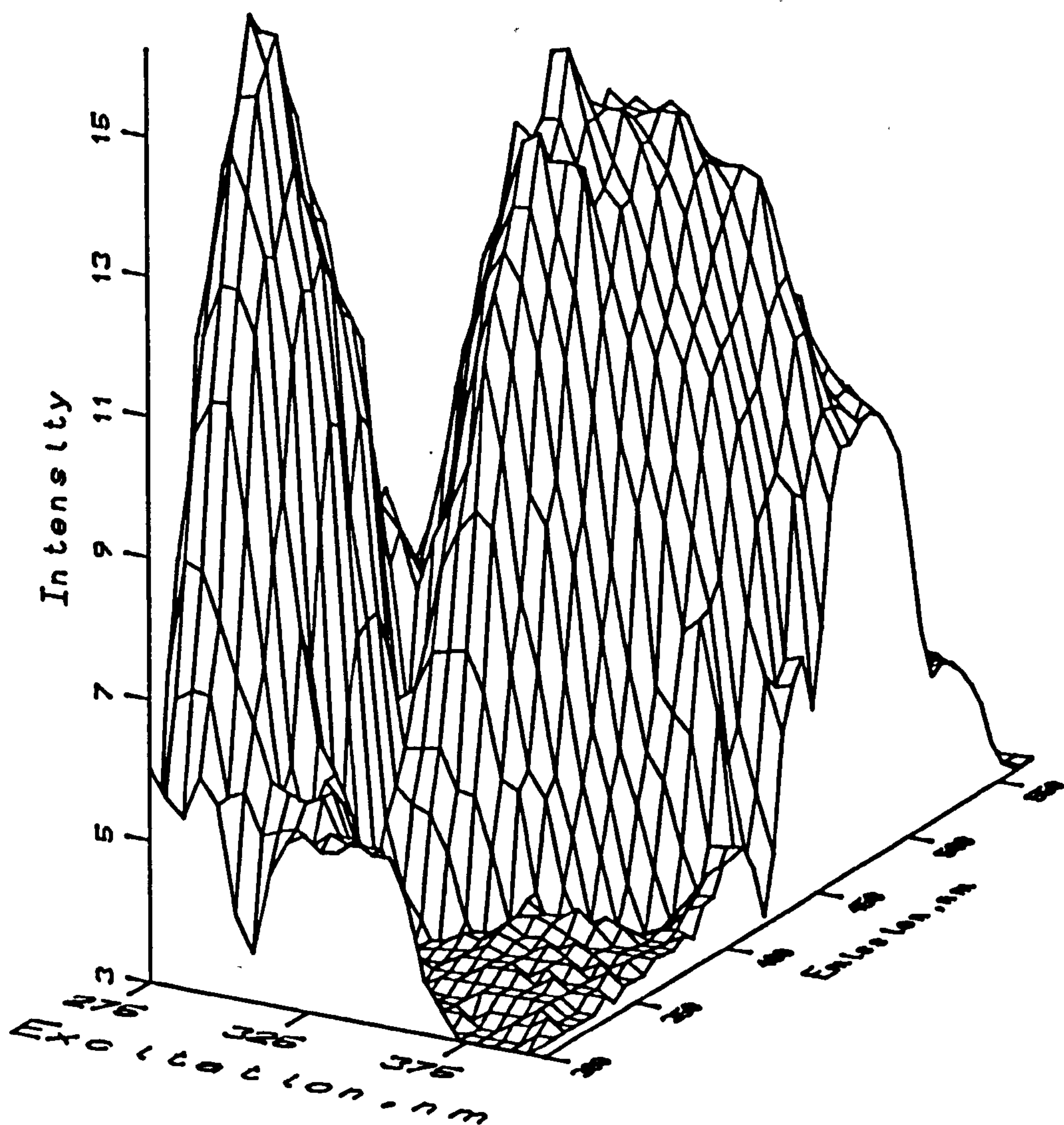


Figure 2.13 Three-dimensional plot of corrected fluorescence intensity from Merino wool in excitation - emission space [220].

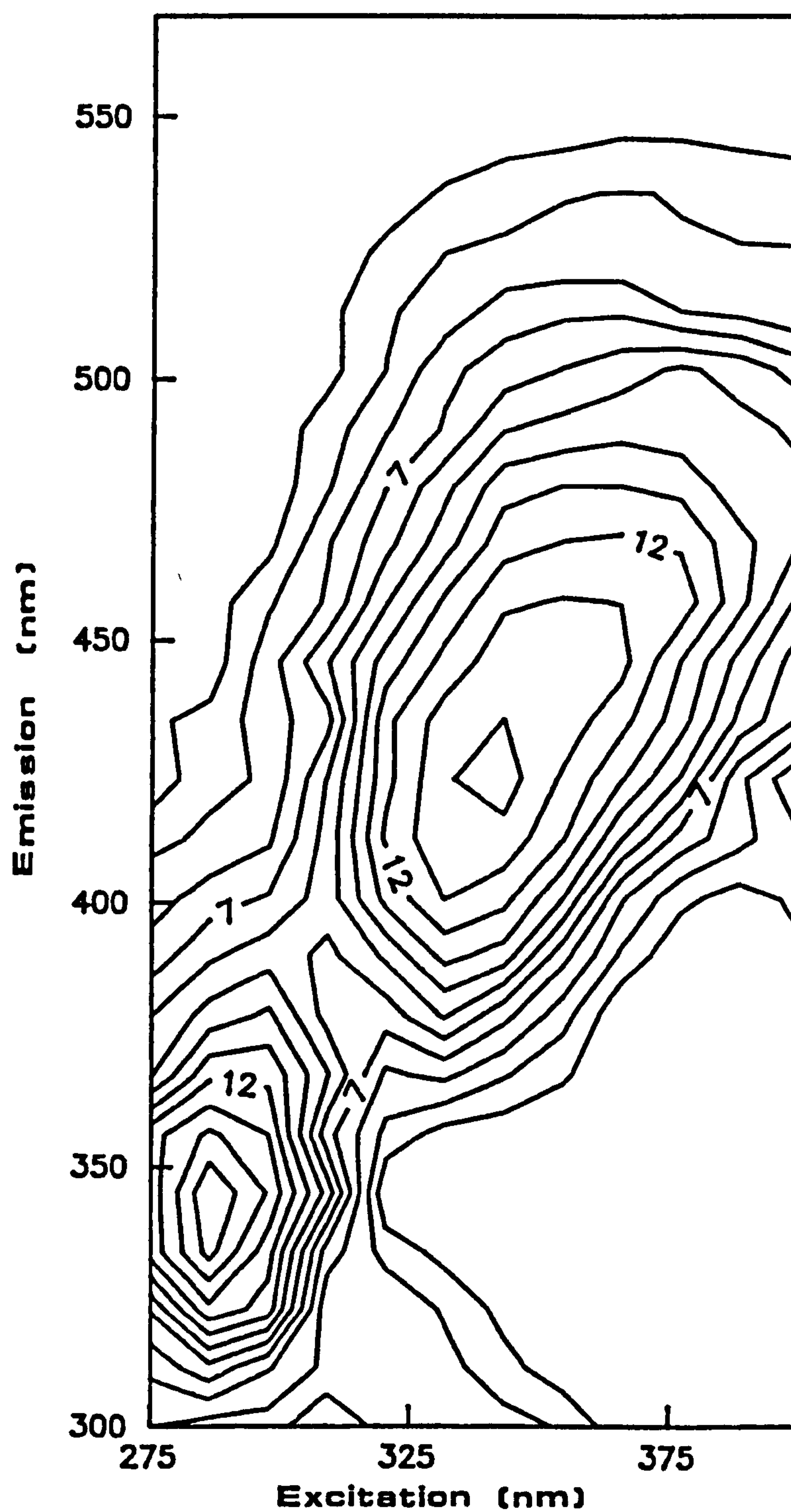


Figure 2.14 Contour map of the corrected fluorescence intensity of Merino wool in excitation - emission space [220].

Different studies have found different maxima for the same exciting wavelength e.g. for exciting at 320 nm, maxima at 402 nm [118] and approximately 420 nm [215] have been obtained. This is not surprising when it is remembered that wool is a biological composite with a non-unique composition (see Section 2.3). Indeed there is some variation in the fluorescence maxima of different types of wool [214]. Fourteen different types of wool were tested by excitation at 360 nm, and the emission maxima found to vary between 425-440 nm.

Temperature does not affect emission maxima [210,218,219]. Heating from 20°C to 90°C lead only to some broadening of the spectra [210], while no substantial (<5 nm) shift of the fluorescence spectrum was observed over the temperature range 300K to 80K (27°C to 193°C) [218]. Heating at 115°C for up to 5 days also caused no substantial change [219]. Similar spectra have also been obtained for different positions along a wool fibre [217], indicating that weathering does not affect the emission maxima.

The effect of water, if any, is minimal. It has been found that drying shorn lamb fibres caused a red shift of 2 nm [214]. However, it has also been observed that adsorbed water did not affect the fluorescence spectrum of cloth [213].

Treatment of wool with Blankit D [118], hydrogen peroxide [118,125,190,214], reduction/alkylation [180], chlorination [190], esterification [180], mineral acid [180], sulphamic acid [216], UV [118,214] and thiourea/formaldehyde [125] cause little, if any, change in the emission maxima. Persulphate oxidation [188] causes a 10 nm red shift (to a relatively small fluorescence emission).

Consequently, the fluorescence emission spectrum of wool is dependent on the excitation wavelength but almost independent of the treatment it receives.

2.5.2.2. Factors influencing intensity

Temperature affects the intensity of fluorescence emission. The fluorescence yield shows only a modest decrease (about half) from 130K to 300K [218] (see Figure 2.15). However, heating from 20°C to 90°C (293K to 363K) leads to a steady

decrease in fluorescence intensity, such that at 90°C the fluorescence intensity is reduced by a factor of about 20 [210]. At temperatures above 100°C (373K) chemical destruction of the tryptophan in wool is found to occur [210]. Schäfer has found that heating wool top at 115°C (388K) for 5 days led to an increase in fluorescence intensity of approximately three fold [219].

It has been known since at least 1927 that light exposure effects the fluorescence of wool [202] (see Section 2.5.1). Then it was found that irradiation by sunlight destroyed the fluorescence while at the same time it was observed that tips of fibres fluoresced differently from the rest of the fibre (UV excitation). In 1943 it was observed that exposure of wool for 64 hours in a Fadeometer caused the fabric to become whiter having a duller and more violet fluorescence (UV excitation). Only in the last few years has further observations been reported, some of which contradict each other.

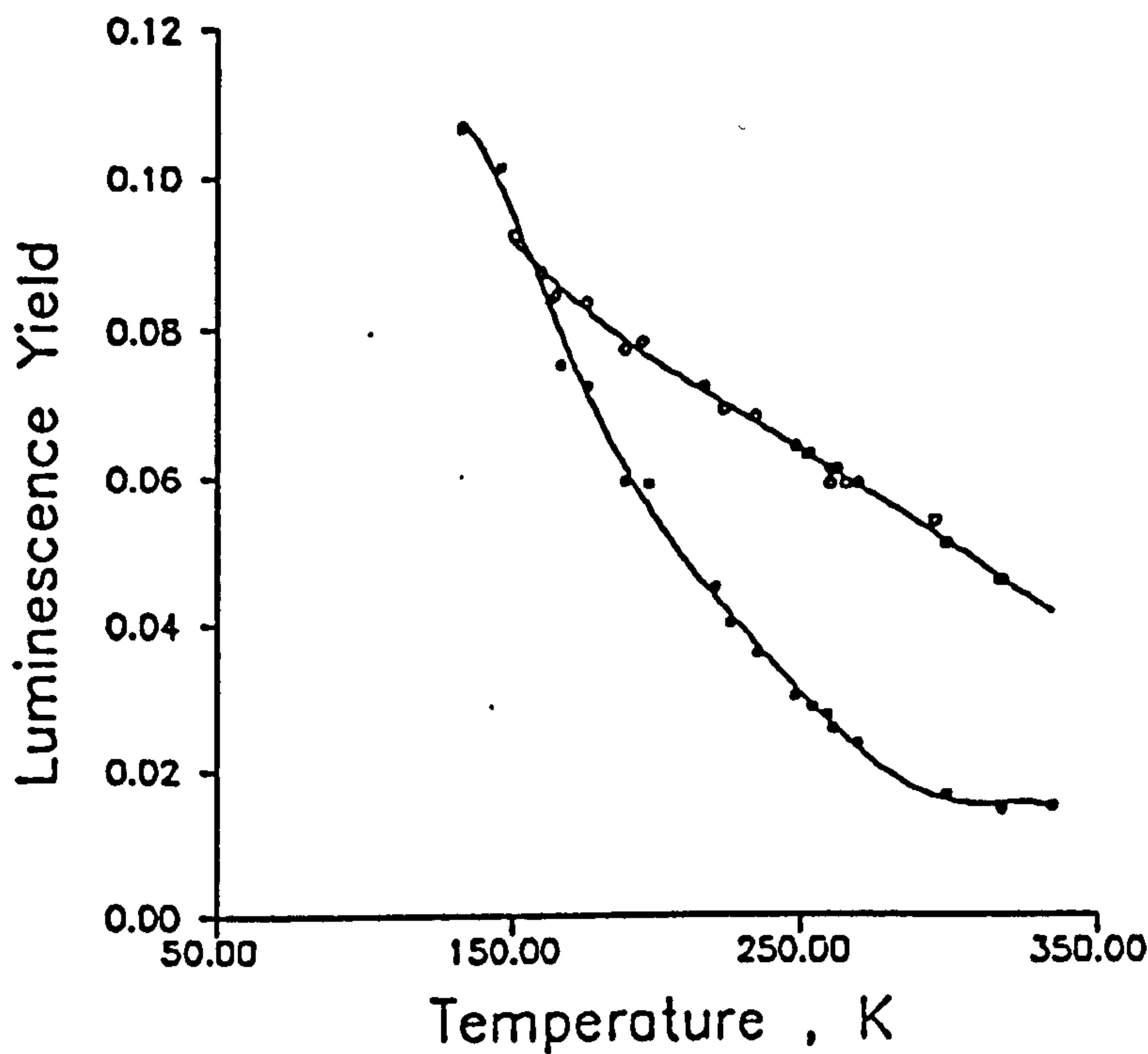


Figure 2.15 Effect of temperature on absolute fluorescence and phosphorescence yields of wool [218].

- Absolute fluorescence yield
- Absolute phosphorescence yield

King [214] found that irradiation of challis for 16 hours with UV fluorescent tubes (having a maximum emission at approximately 300 nm) caused oxidatively bleached and unbleached fabric to decrease by 10-20% in fluorescence intensity if excited at 290 nm (and measured at 335 nm) but remain the same if excited at 360 nm (and measured at 430 nm). Jones [118] however, found different results using 350 nm fluorescent tubes for 24 hours and irradiating the samples whilst wet. Over the excitation range 340 to 380 nm, untreated wool fabric lost approximately one third of its fluorescence intensity, and peroxide bleached wool lost nearly two thirds, while Blankit D treated wool remained about the same, showing that it was apparently photostabilized. Persulphate oxidised wool, measured only at the excitation wavelength of 380 nm, remained unchanged. However, this treatment causes extensive yellowing and Jones thought that the existence of an inner filter effect, where the blue fluorescence would be reabsorbed by the yellow wool, could not be discounted. Cameron and Pailthorpe [216] found that irradiation of untreated and sulphamic acid-treated wool with an artificial light source for 4 weeks led to the fluorescence being reduced to virtually zero.

Schäfer, during the course of this investigation, reported on the effects of light - both natural and artificial - on wool [217,219]. Using a standard Australian Merino top and irradiating with a Xenotest 150 (using the filter system 4 IR/3 window glasses) a marked increase in fluorescence intensity was observed (Table 2.8). Recording emission spectra at different positions along a wool fibre (from a 1 year old Texel sheep) Schäfer found that the intensity increased approximately four fold from root to tip. Additionally, the emission spectra were similar. She also investigated the effect of 'photobleaching' [219]. Exposing wool to daylight behind window glass led initially to photobleaching and a lowering of fluorescence intensity. Continued exposure led to the onset of photoyellowing being detected which was accompanied by an increasing natural fluorescence.

Length of Irradiation(h)	Fluorescence Intensity (at 440 nm)	Yellowness Index	Trp (mol%)	CySO ₃ H (mol%)	Cys (mol%)
0	0.113	19.97	8.32	0.180	7.096
22	0.362	23.76	3.45	-	-
70	0.393	26.04	1.39	-	-
120	0.607	29.61	1.71	0.802	2.762
245	0.947	34.12	-	-	-
500 ^a	1.382	49.56	-	-	-

^aa different Australian Merino top was used.

Table 2.8 Analysis of the effect of light (Xenotest 150) on wool, restated from [217].

Various chemical treatments are known to affect the fluorescence of wool. The most widely studied has been oxidative bleaching. Leaver [125] found that this caused an increase of 40% on excitation at 290 nm. King [214] found a 10% increase in the fluorescence of challis. Jones [118] obtained a similar value of 8% for three different wavelengths of excitation while Collins *et al.* [190] found a decrease on exciting at 300.5 nm but a doubling on exciting at 375 nm. Stewart [9] qualitatively observed no significant change in fluorescence when the treatment was carried out at room temperature, but saw a significant increase exciting at 365 nm or 350-400 nm) when the oxidation was carried out at 80°C. Schäfer [217] also found an increase in fluorescence with oxidative bleaching, but not as large as that caused by light. Microscope examination of single fibres from an oxidatively bleached wool top showed that the fluorescence was inhomogeneous, with the proportion that are more intense being larger than in untreated wool top. Reductive bleaching of wool was also found to increase the fluorescence, comparable to oxidative bleaching. The increase in fluorescence on oxidative bleaching is attributed to the conversion of quenching cystine to non-quenching cysteic acid. Subsequently, Schäfer reported again on the effect of bleaching [219]. Then oxidative bleaching was found to cause a small increase in fluorescence while reductive bleaching and chlorination both caused a large decrease in fluorescence intensity.

Jones [118] found that treatment with Blankit D caused wool to lose approximately half of its fluorescence over the excitation range 360 to 380 nm. Jones also found that persulphate oxidised wool, measured only at the excitation wavelength of 380 nm, lost most of its fluorescence. Leaver [125] observed a marked decrease in the fluorescence of untreated and oxidatively bleached wools upon treatment with thiourea/formaldehyde. Cameron and Pailthorpe [216] observed that sulphamic acid-treatment caused the fluorescence of wool to decrease markedly which they decided was due to some form of masking, filtering or quenching resulting from the treatment. Collins *et al.* [190] found that chlorination of wool led to a decrease in fluorescence on exciting at 300.5 nm but gave a very marked increase on exciting at 375 nm. Stewart [9] found that the presence of metal ions (copper and iron) did not seem to have a significant effect on wool fluorescence as treatment with the strong chelating agent, oxalic acid, which removes any metal ions, did not affect fluorescence.

Bhatnagar and Gruen [180] examined the fluorescence characteristics of a number of chemically modified wools. Acylation was found to cause a decrease in fluorescence with short-chain and bifunctional acylating agents exerting a greater quenching effect on the fluorescence than long-chain acylating agents.

Esterification of wool was accompanied by an increase in fluorescence, the effect decreasing as the chain length of the alcohol employed was increased. They attributed the enhanced fluorescence to more rapid dissolution of the (heat induced) yellow pigment by the alcohols of lower molecular weight and, to lower rates and extents of esterification as the molecular size of the alcohol is increased.

Acid was also found to affect the fluorescence. Dilute acid enhanced the emission but prolonged treatment, increased acid concentration or higher temperatures all lead to reduced fluorescence. The absence of oxygen and water affects the fluorescence of wool. Ghiggino *et al.* [179] observed that the fluorescence emission of wool at room temperature decreased after equilibrating in dry nitrogen.

There is evidence that the disulphide bonds of wool cause considerable fluorescence quenching. Reduction of the disulphide bonds in wool by treatment with tri-n-butylphosphine was found to double the original fluorescence [180,181] indicating that the presence of the disulphides was responsible for at least 50% quenching of wool's fluorescence. Bhatnagar and Gruen [180] found that subsequent alkylation of the thiol groups either decreased or further enhanced the fluorescence, depending on the substituent introduced, but always gave a higher fluorescence than the untreated sample. (They had earlier observed a two fold increase in fluorescence intensity when a high sulphur fraction extracted from wool had been fully reduced and alkylated (one sample only) [211]). Leaver [181] however, found that subsequent alkylation removed the enhancement achieved by the reduction process and attributed this loss of fluorescence to conformational changes in the fibre as a result of the chemical treatment.

Cowgill proposed that the peptide bond could be quenching the fluorescence of soluble proteins [123]. This was based on his findings that the fluorescence quantum yields of a number of tyrosine and tryptophan derivatives and peptides in solution were significantly lower than the corresponding values for the free amino acids. However, it has been shown [221] that the quantum yield of fluorescence of tyrosine and tryptophan derivatives and peptides in PVA film has the same fluorescence as the free amino acids. Consequently, it would appear unlikely that the peptide bond causes significant fluorescence quenching in wool keratin [65].

Factors in nature as well as sunlight also have an effect on the fluorescence of wool. Schäfer [217] stated that the fluorescence of wool depended on the wool's origins (sheep breed, wool quality - staple length, fleece position, bacteria infection, dung and urine etc) and the treatment of the wool (before any artificial chemical treatment). Schäfer found that the melanin in pigmented wool fibres acts as a fluorescence quencher causing the melanin granules to show up as dark places. She also found that urine discoloured fibres show different fluorescence characteristics from pigmented and raw white wool, enabling the three to be distinguished from one another.

2.5.2.3 Other Physical Measurements

Smith *et al.* [213] found that the fluorescence decay could best be described by a single exponential, which gave a lifetime of the fluorescence decay as 6.9 ± 0.6 ns (excitation was at 270 nm and the emission detected at 330 nm).

Melhuish and Smith [218] decided that the radiative processes associated with relaxation of the fluorescent singlet state are independent of temperature and calculated the activation energy for the relaxation of the excited singlet state of tryptophan in wool as a comparatively small 580 cm^{-1} .

Fluorescence quantum yields have been obtained for a number of conditions (see Table 2.9 and Figure 2.15) [84,179,218,222]. Decreasing the temperature causes the quantum yield to increase, while removal of room air causes the quantum yield to decrease.

Excitation Wavelength (nm)	Quantum ϕ_F	Yields ϕ_P	Phosphor-escence Lifetime(s)	Conditions	Reference
280	0.20	0.13	4.76	77K	179
	0.050	0.0135	0.450	room temp dry N ₂	
	0.082	0.0018	0.080	room temp, lab atmosphere	
280	0.054	0.024	-	295K ϕ_F ; 262K ϕ_P ; evacuated	218
	0.094	0.107	-	130K evacuated	
290	0.022	-	-	corrected, room temp	184
290	-	0.13	4.76	77K	62
		0.0135	0.45	20°C, dry N ₂	
		0.0018	0.08	20°C, room air, 50% r.h.	
350	-	0.060	0.235	77K	62
		0.0073	0.160	20°C, dry N ₂	
		0.00074	0.020	20°C, room air, 50% r.h.	

Table 2.9 Quantum yields of luminescence and phosphorescence lifetimes of wool (F = fluorescence, P = phosphorescence).

2.5.3 Phosphorescence of Wool

2.5.3.1 Spectral Characterisation

The phosphorescence emission of wool, like the fluorescence emission, is dependent on the excitation wavelength. Emission spectra have been obtained over the excitation range of 280 to 360 nm [62,90,179,184,215,216]. The excitation wavelengths together with their emission maxima are listed in Table 2.10. Lennox *et al.* [90], as well as Nicholls and Pailthorpe [62], have commented on the phosphorescence emission maxima varying with the exciting wavelength. The latter have attributed this to the presence of two phosphorescent species: the first (tryptophan) emitting at approximately 440 nm when exciting at 290 nm and the second (unknown) emitting at 500 nm when exciting at 350-400 nm. Exciting in the intermediate-wavelength range of 290-340 nm results in a combination of emission from both species.

Treatment of wool with sulphamic acid and/or an artificial light source [216] cause little change in the phosphorescence emission maxima.

Excitation Wavelength(nm)	Emission Maxima(nm)	Conditions	Reference
280	approx 430	77K, corrected	179
280	approx 440	room temp, corrected	179
280	approx 450	20°C, N ₂	216
290	435	room temp.	62
approx 300	approx 460	77K	90
330	approx 470	77K, corrected	215
340	480	room temp, N ₂	184
350	500	room temp.	62
360	505	77K, corrected	215

Table 2.10 Variation of phosphorescence emission of wool with variation in excitation.

2.5.3.2 Factors Influencing Intensity

Temperature affects the intensity of phosphorescence emission [210,218]. The phosphorescence yield shows a marked decrease with increasing temperature [218] (see Figure 2.15).

Little has been reported on the effect of light on the phosphorescence of wool. Millson in 1943 [209] was the first to investigate this phenomenon (see Section 2.5.1). He found that exposure of wool for 64 hours in a Fadeometer caused the colour of the phosphorescence to become yellower, but with about the same rate of decay. Cameron and Pailthorpe [216] found that irradiation of untreated and sulphamic acid-treated wool with an artificial light source for 4 weeks led to a modest reduction in phosphorescence intensity.

The presence of water and oxygen have an effect. This is considered in Section 2.5.3.3.

Various chemical treatments are known to affect the phosphorescence of wool. Cameron and Pailthorpe [216] found that treatment with sulphamic acid caused a decrease. This they attributed to the lower tryptophan content in the treated wool probably arising from the curing process involved. Leaver [183] found that commercial scouring increased phosphorescence intensity by 60% and peroxide bleaching caused the intensity to approximately double. He also found that woven fabrics phosphoresced more strongly than loose wool samples indicating that wool processing treatments have an effect.

Natural factors may also have an effect. Leaver [183], using the fleece of pen-grown sheep, found that Lincoln fibres had a slightly larger phosphorescence than Merino fibres.

2.5.3.3 Other Physical Measurements

The phosphorescence lifetime of wool has been the subject of much study [62,179,181,183,209,210,215], most of which has been concerned with the room temperature phosphorescence. Konev [210] examined the decay kinetics of the room temperature phosphorescence and found both an intense short-lived component and a longer-lived weak component. The first, intense, component decayed rapidly, almost exponentially, in the first 30 seconds and it was observed that: warming to 75°C decreased the intensity (10 fold); in the case of dry wool oxygen caused a decrease and that moistening of the sample in the absence of oxygen led to an increase in intensity. The second, weak, component decayed slowly over many minutes and it was observed that: warming to 75°C increased the intensity (exponentially, giving an activation energy for wool of 21.5 kcal mole⁻¹ (90.0 kJ mole⁻¹)); in the case of dry wool oxygen had no great effect and that moistening of the sample in the absence of oxygen led to a reduction in intensity. Konev attributed the short-lived component to direct emission from the tryptophan triplet and proposed that the longer-lived component was due to recombination of a photoejected electron with the tryptophan radical cation.

Ghigginio *et al.* [179] have also examined the room temperature decay kinetics of phosphorescence. Exciting at 280 nm they found that under normal atmospheric conditions the phosphorescence decayed by first-order kinetics with a lifetime of 0.08 s (Table 2.9), while under dry nitrogen its lifetime was 0.45 s and decayed at an apparent second-order rate. They concluded that under dry nitrogen the rate-determining step is a radiationless tryptophan triplet-triplet quenching but in the presence of oxygen (and some moisture) the triplet state of tryptophan is quenched by oxygen with the possible formation of singlet oxygen.

Nicholls and Pailthorpe [62] found two quite distinct phosphorescence species by studying the excitation wavelength dependence of the phosphorescence of wool. With an excitation wavelength of 290 nm the emission (peak 435 nm, lifetime at room temperature in dry nitrogen 0.45 s (see Table 2.9)) was attributed to the triplet state of tryptophan. This was based on the good agreement of its spectral line shape with the emission from tryptophan in a PVA film under the same

conditions. When excited by 350 nm radiation a second phosphorescent species (peak emission 500 nm, lifetime 0.16 s) was observed. This species could be detected for excitation wavelengths of up to 400 nm. In the intermediate wavelength range of 290-340 nm the long-lived emission from wool was found to be a combination of both these emissions. Thus, although the donor-acceptor conditions appear to be fulfilled, there is no energy transfer from tryptophan in its triplet-excited state to the other phosphorescent species. The source of this latter emission is not clear but may be the same species which is responsible for the enhanced absorption of wool in the 330-360 nm range [65].

Leaver [183] re-examined the room temperature phosphorescence decay of wool. He found that, for excitation at 290 nm, the emission consisted of two separate components which decay exponentially with lifetimes of 0.18 ± 0.03 and 1.45 ± 0.25 s. The former was attributed to the species with a maximum absorption in the 330-350 nm region, while the long-lived species was attributed to the triplet state of tryptophan.

Low temperature (77K) phosphorescence of wool has also been observed [62,181,215]. Leaver [181] exciting at 290 nm found that this was due to a relatively weak long-lived tryptophyl component ($\tau = 4.8$ s) superimposed on a more intense component having a much shorter duration.

Hence, it appears that the phosphorescence of wool is made up of three components. The first, having a lifetime of about 0.2 s, arising from an unknown species; the second, having a lifetime of about 1.5 s, arising directly from tryptophan (and not observed when exciting at 350 nm); and the third, having a lifetime of many seconds, arising indirectly from tryptophan.

The presence of air causes a reduction in the phosphorescence lifetime [62,179,181] due to the presence of oxygen (a quencher). Additionally, Leaver [181], found that the source and treatment of the wool affected the lifetime particularly that of the shortest-lived component.

Phosphorescence quantum yields have been obtained for a number of conditions (see Table 2.9 and Figure 2.15) [62,179,218]. Decreasing the temperature causes the quantum yield to increase, as does the removal of room air. Presumably, under dry nitrogen, the plasticiser water is removed, the structure becomes far more rigid and intersystem crossing is relatively enhanced [179]. The first two components of the phosphorescence of wool have different quantum yields. The second (tryptophan) has a quantum yield of about double that of the first (unknown).

2.5.4 Origins of the Luminescence

The origins of the luminescence of wool, as far as they are known, have largely been explained in Section 2.4.7, and can be summarised thus:

For wavelengths of less than 320 nm, the energy absorbed by the wool is due to the amino acids tyrosine and tryptophan. All the energy absorbed by the tyrosine is efficiently transferred to tryptophan so that at room temperature the fluorescence and phosphorescence emission from wool is entirely due to tryptophan [179]. Wool absorbs the 320-400 nm region of sunlight by virtue of the presence of a chromophore that is as yet unidentified.

A number of compounds have been proposed as causing this (blue) fluorescence. None are solely responsible but each may contribute slightly.

Dityrosine is known to occur in wool and wool proteins [9,56,223,224]. The absorption spectra of dityrosine shows a typical protonation equilibrium, with absorption maxima at 284 nm in acid medium and at 316 nm in alkaline medium [9]. On excitation at 315 nm (at pH 9.5) fluorescence is observed with an emission maxima at 406 nm [9]. Peroxide bleaching [9], artificial irradiation [9,224] and heating [224] all cause increased levels of dityrosine in wool. As the excitation maxima of the main chromophore responsible for visible fluorescence has been found to be 370 nm, it indicates that dityrosine is not responsible [190].

Many of the other proposed compounds are derivatives of tryptophan. Destruction of the tryptophan in wool is known to be related to photoyellowing (see Section 2.4.7) and the corresponding increase in fluorescence (Table 2.8). Kynurenine, a recognised oxidation product of tryptophan, has been identified as a product of photodegraded wool [165]. L-kynurenine has also been identified as causing the fluorescence of the hair of rats [225,226]. Kynurenine is a weak emitter of fluorescence, with an emission maxima at 480 nm on excitation at 365 nm [227]. However, irradiation of wool in the range 360 to 370 nm causes emission maxima in the range 430 to 450 nm [118,214,215], indicating that kynurenine is not responsible for the visible fluorescence of wool.

The visible fluorescence of wool has been attributed to the excitation of N-formylkynurenine (NFK) residues, formed by photodegradation of tryptophan residues [215]. NFK has been detected in eye lens protein and egg-white lysozyme [227-229]. The absorption band of aqueous NFK extends to about 350 nm, with the longest wavelength peak at 320 nm as well as other peaks at 230 and 260 nm [230]. The fluorescence of NFK is relatively weak, with a remarkable dependence on the polarity of the medium. Exciting at 260 or 320 nm, the fluorescence maximum is at 430 nm in polar media and at 510 nm in non-polar environments [230]. However, exciting wool at 265 nm produces a fluorescence maximum at about 340 nm [210], while exciting wool at 320 nm produces a different maximum, at 402 nm [118] to about 420 nm [215].

Even though none of the above mentioned compounds are solely responsible for wool's visible fluorescence they, and other compounds, may still contribute to the fluorescence. Firstly by being fluorescent themselves and secondly by acting as photosensitisers. For example, NFK is a well known photosensitiser [231-235], and could be contributing to the formation of the unknown chromophore(s).

The present work, at least in part, has been directed towards a more detailed examination of the fluorescence behaviour of wool. By providing a better understanding of the underlying chemical processes occurring in photoyellowing the development of better protective agents will be assisted.

2.6 DIFFUSION

The study of the movement of reagents, for example dyes, into wool fibres is the subject of much on going research. The successful dyeing of wool fibres involves both satisfactory exhaustion of dye from the dyebath onto the fibre, and adequate penetration (diffusion) of the dye within the fibre [236]. The practical advantages of complete dye penetration are maximum wet fastness, light fastness and colour yield. With many dyes uniform and complete penetration is often difficult to achieve.

2.6.1 Reagent Diffusion

The diffusion of dyes and other reagents into the wool fibre has recently been reviewed [236,237]. The reagents, after diffusing through the solution to adsorb at the surface of the fibre, diffuse into the fibre. The rate of penetration generally increases with increasing temperature and fibre swelling, but decreases with increasing size of the reagent and crosslink density of the fibre (for example, the number of disulphides) [237]. The extent of penetration increases with time before levelling out to an equilibrium distribution. The distribution pattern can vary depending on the reagent and the conditions used. Other important factors in the mechanism of penetration into the fibre are the molecular structure of the reagent (for example, its hydrophobic/hydrophilic nature), the crosslink density of the different morphological regions of the fibre, and pretreatments to which the fibre had been subjected [238-242].

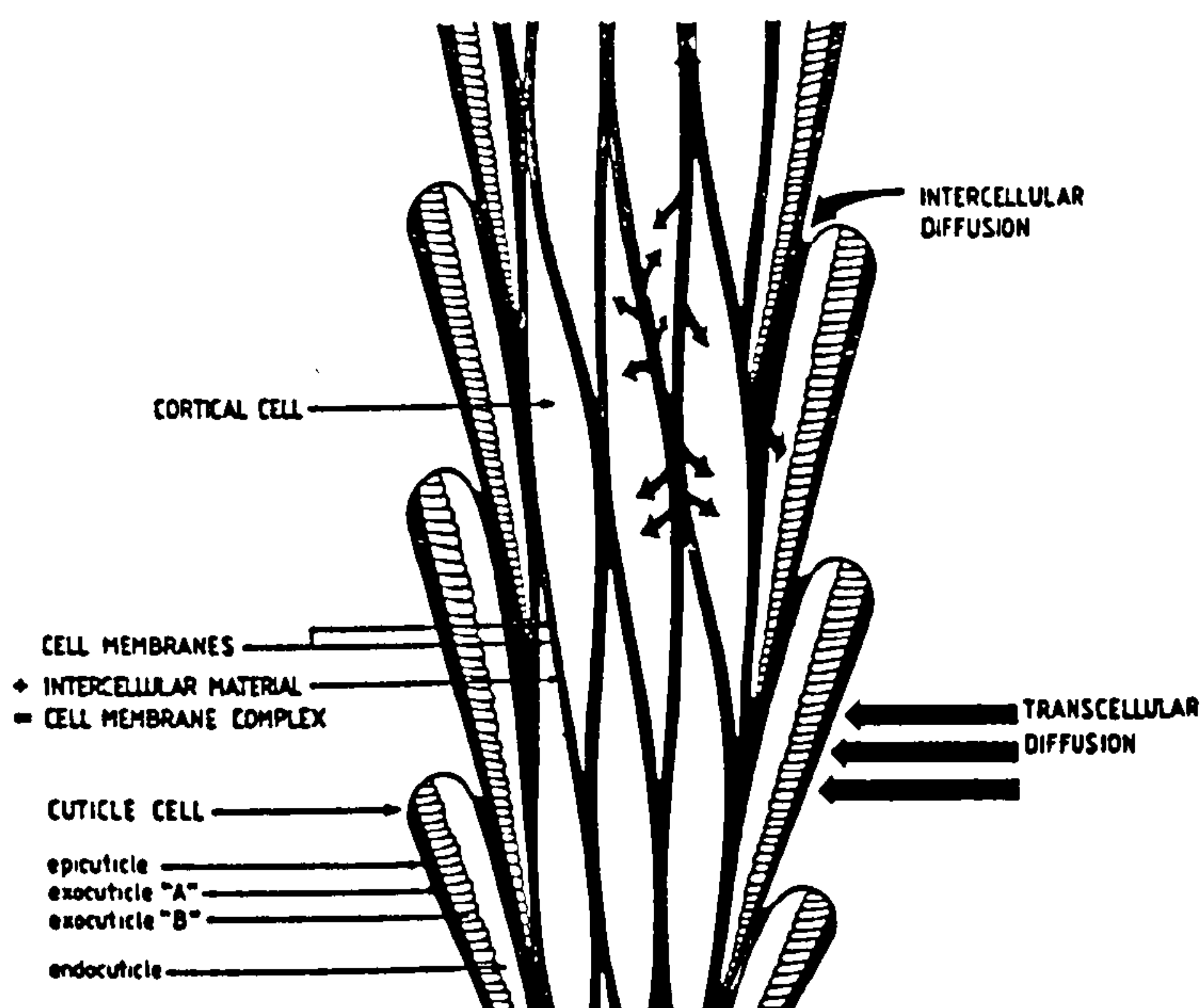


Figure 2.16 Schematic diagram of a keratin fibre showing the transcellular and intracellular routes for the penetration of reagents [237].

Two pathways exist for diffusion of reagents into the fibre (Figure 2.16) [237]:

1. Transcellular Route across the cuticle and cortical cells which vary in crosslinked densities. This is probably the preferred route for small molecules, especially after damage to the highly crosslinked exocuticle layer.
2. Intercellular Route through the presumed low crosslinked intercellular material prior to penetrating the cell membranes into either the endocuticle or the microfibril/matrix complex. This is generally the preferred route for diffusion [236,243-247].

This has been established using either heavy metals and transmission electron microscopy [236,243,247] or fluorescent molecules and fluorescence microscopy [236,244-246].

2.6.2 Effect of Solvents

Recent investigations into the diffusion of a dye (Rhodamine B) into the wool fibre [244,246] found that the distribution pattern of the dye was strongly dependent upon the solvent composition. Selective staining of the cell membrane complex occurred in an anhydrous solvent system, whilst aqueous systems caused either the cell membrane complex associated with the orthocortex or the entire orthocortex to be preferentially stained. Water alone caused even staining. (Similar results have been observed using human hair [248]). Thus, by judicious choice of solvent system and treatment time, it was found that dyes could be directed into different histological components of the fibre. This is a specific consequence of the use of different solvents.

Bradbury and Leeder [249], and Watt [250] demonstrated that there was a gradation in the rate at which solvents such as alcohols and carboxylic acids enter the wool fibre - the rate being determined largely by the molecular size of the solvent. The classification 'non-swelling' solvents has been used to describe those solvents which do not enter the wool fibre [238]. Even solvents such as benzene and heptane which enter the wool fibre can still be regarded effectively as 'non-swelling' solvents as they enter at a much reduced rate compared to the low molecular weight alcohols. Tetrachloroethene, toluene, dimethylformamide and t-butanol are also known 'non-swelling' solvents [238,251,252]. Ethanol and chloroform/methanol swell the fibre and have consequently been used to extract the internal lipids from wool [252]. The choice of solvent used in the analysis of solvent-soluble matter in wool can therefore have a large effect on the values obtained [252-255].

Traditional 'non-swelling' solvents have been found to readily enter the fibre when a small amount of a 'swelling' solvent is also present. Thus, methanol increases the uptake of tetrachloroethene, toluene and dimethylformamide by wool, the effects being greater with the more hydrophobic solvents [238]. The moisture content of wool also has an effect [256]. A small amount of water has been found to increase the uptake of tetrachloroethene [251].

This has led to the proposal that compounds such as ultraviolet absorbers and moth proofing agents could be applied to wool from organic solvents under swelling conditions [239]. Some nonionic compounds [239] and photosensitising dyes [244] are among those already investigated.

The differing effects of solvents provides a useful way of isolating separately the sebaceous, surface and internal lipids of wool [237]. Cleaning of greasy wool with a solvent combination such as t-butanol/heptane followed by water removes the sebaceous lipids, dirt, sweat and other skin secretions (see Chapter 3). Once these surface contaminants have been removed, the surface lipids (fatty acids, cholesterol) may be isolated by treating the clean wool with t-butanol/t-butoxide under non-swelling conditions [257]. (These surface lipids could more readily be regarded as part of the wool fibre as they seem to have been cleaved from the epicuticle). Treatment of the wool with chloroform/methanol then removes the unbound internal lipids such as fatty acids and sterols, and trace amounts of glycerides, sphingolipids and glycolipids (see Section 2.6.3).

2.6.3 Internal Lipid Composition

The analysis of some or all of the internal lipids of the wool fibre is the subject of much recent investigation [38,254,255,258-274]; some of which [38,258-263,274] is more thorough than the rest. While the analyses are in qualitative agreement with each other, the actual values show very wide discrepancies. For example, Crabtree *et al.* [260] found that 72% of the internal lipids was cholesterol and 12% fatty acids, while Schwan *et al.* [263] found a sterol content of 25% and a free fatty acid content of 24%! This arises mainly because internal lipids are a natural product (and so would be expected to show a variation between samples) and partly because the analytical conditions are not always consistent e.g. different solvents extract different amounts and components, of the lipids [28].

The largest major component of the internal lipids is sterols - notably cholesterol and its precursor, desmosterol [267], along with small amounts of the oxidation products 7 α - and 7 β -hydroxycholesterols [260]. It has been found that although the amounts of cholesterol and desmosterol vary between samples the ratio is relatively constant at about 2:1 (cholesterol : desmosterol) [267].

The smallest major component of the internal lipids is free fatty acids. Although many saturated and unsaturated acids are present, most are only in minor quantities. The major acids present are hexadecanoic, octadecanoic and octadecenoic acids [259,260,263,270,273]. There is an almost complete lack of iso- and hydroxyfatty acid components [263].

A number of components in small or trace amounts have been reported. Cholesterol esters are present in small amounts [259,260,262,263], as are mono-, di- and triglycerides [260,262,263]. Phospholipids are present in trace amounts [259,265,269]. Glycolipids [268], ceramides [254,262,269,272] and cerebroside [262,269] have also been identified. However, no hydrocarbons have been found [263].

Comparison of the composition of wool wax (Section 3.2) with the composition of the internal lipids indicates that the two are separate entities, showing that the internal lipids did not originate in the wool wax [258-260,263,265].

The proceeding sections have shown that compounds can easily enter the wool fibre under artificial ('swelling') conditions but that diffusion into the fibre does not normally occur in nature (otherwise the internal lipids and the external wool wax would not be separate entities). However, this is not to say that migration into the wool fibre cannot occur in nature. Chipalkatti *et al.* [275] have shown that (unidentified) pigments present in the suint are responsible for canary coloration in Indian raw wools (see Section 3.3.1). Whether this is an isolated case, and if not, what consequences it has, are not known at present.

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CHAPTER 3

INTRODUCTION TO WOOL YOLK

3.1 PREFACE

The natural by-products of the fleece have been described by many different terms. These are often used differently by different authors. In the present work the relevant definitions of Truter [1] are used and are given below. It should be noted that it is not always possible to know if the authors cited conform to this nomenclature.

'Yolk comprises all the physiological products of the fleece except the fibre. It consists of two main fractions, the wool lipid and the suint.

Wool wax, occasionally referred to as the wool lipid, is the lipid material in the fleece that *can* be extracted with the usual fat solvents. It refers specifically to the lipid excretion of the sheep's skin. Because of the heterogeneity of the wax, different solvents may preferentially extract different components...

Suint is the water-soluble material contained in the fleece. (Some workers would add the rider "after the wax has been extracted."...)

Wool grease, after wool has been scoured, i.e. washed, the wool lipids remain suspended in the wash-waters through the agency of the detergent. This emulsion also contains suint. Attempts to recover the lipid usually give products contaminated with detergent and suint. For this reason the product is called wool grease as distinct from wool wax ...

Lanolin is wool grease that has been subjected to a combination of refining processes designed to lighten its colour, improve its odour and to reduce the free acid content ...'

Before embarking upon a description of the yolk constituents, i.e. wool wax and suint, a brief outline of the histology of the sheep's skin will contribute to a clearer understanding of the topic.

Figure 3.1 [1] shows the longitudinal section of a wool follicle. The sebaceous gland is believed to produce the wax [1,2] and the sudoriferous gland the suint [1]. As the opening of the sebaceous gland is placed below that of the sudoriferous gland, the wax is deposited directly onto the growing fibre while the suint is deposited over the wax. This is the situation for the primary fibres; the secondary fibres differ in that they do not usually have a sudoriferous gland.

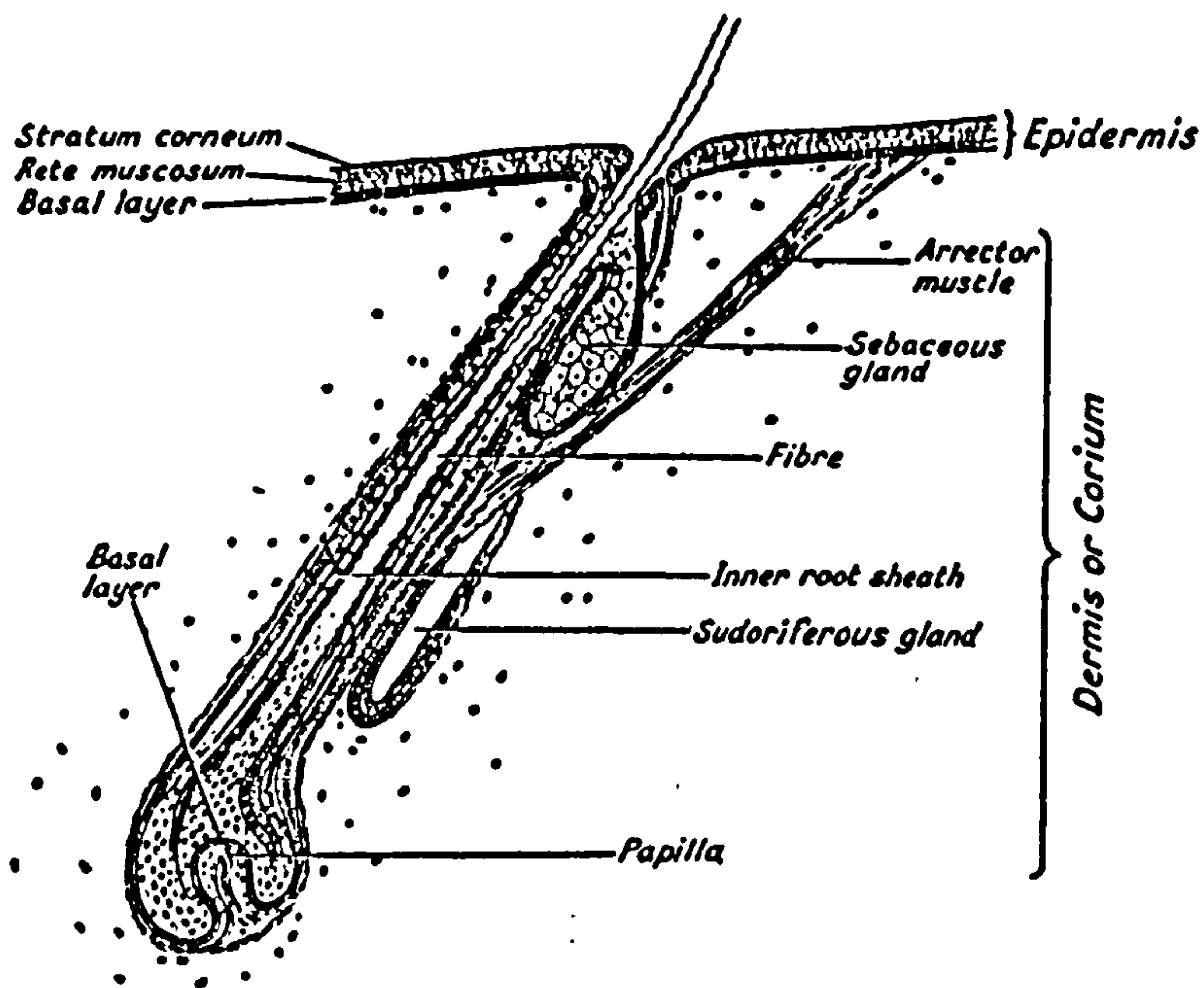


Figure 3.1 Longitudinal section of a wool follicle [1].

The wool fibre as well as having the natural constituents of the yolk (wool wax and suint) also acquires impurities during growth [1,3]. These include sand, dust, burrs, grass, epidermal scurf and moisture.

3.2 WOOL WAX

The amounts of wax and suint occurring in the fleece vary from one breed of sheep to another. Coarse-wooled types invariably have a lower wax index than the fine-wooled types [1]. Table 3.1 shows the relationship between wool fineness and wax content for shoulder samples taken from sheep of five different breeds kept together in the single flock for one year on irrigated pasture [4]. It has also been observed that the wax contents are sometimes different for wools of the same quality, but from different breeds [1].

The distribution of wax is found to vary over the body of the sheep [1]. It seems likely that all sheep have the highest wax index on the back and rump, and the lowest on the shoulder, with the other regions having nearly equal indices.

Other factors which may influence the quantity of wax in the fleece have been considered [1], but generally insufficient experimental observations have been obtained to allow definite conclusions to be reached. The effect of weathering and position along the fibre are dealt with in Section 3.2.2.

The methods used to recover the wool wax from the wool fibre have been reviewed by Truter [1], and by Stewart and Story [5]. Three main methods are used [2]. The first is the solvent extraction method, where the wax is leached out by percolating a suitable solvent through the raw wool and then obtained by subsequently evaporating the solvent. The other two methods involve the scouring of the wool with either soap and alkali or detergent and recovering the wool *grease* by either centrifuging or ‘acid cracking’.

Sheep Breed	Average Fibre Fineness		Wax Content(%) based on		Suint Content(%) based on	
	Diameter (μm)	Quality Grade	Grease Weight	Clean Weight	Grease Weight	Clean Weight
Rambouillet	21.4	64s	13	22.0	13	22.0
Columbia	28.1	56s	11	16.4	10	14.9
Suffolk	34.7	46s	11	18.0	14	23.0
Navajo	37.7	40s	6	8.7	15	21.7
Lincoln	38.5	40s	6	8.3	13	18.1

Table 3.1 Variation of the wax and suint content of wool with its fineness [4].

3.2.1 Composition

Wool wax is a complex mixture of esters of high molecular mass, containing small amounts of free acids and free alcohols [1]. The isolation of the wax esters is so difficult that it is seldom attempted. The usual method of investigation is to hydrolyse the wax and to examine the resultant acids and alcohols separately. Some average physical and chemical data for the more easily separated fractions are given in Table 3.2 [1]. As wool wax is a natural product it is to be expected that the figures show a certain amount of variation. Indeed it is known that the wax composition varies with breed of sheep [6,7] and even in sheep of the same breed [8]. (The effect of weathering and position along the length of the fibre are dealt with in Section 3.2.2.) Consequently, it is not surprising that different investigations find different proportions of the constituents of wool wax.

Characteristic Property	Value Range
Colour	Yellow to pale brown
Density at 15°C (gm.cm ⁻³)	0.94 - 0.97
Refractive index at 40°C	1.48
Melting point (°C)	35 - 40
Free acid content (%)	4 - 10
Free alcohol content (%)	1 - 3
Iodine value (Wijs)	15 - 30
Saponification value	95 - 120
Molecular weight (Rast; in salol)	790 - 880
Proportion of fatty acids (%)	50 - 55
Acids: Melting point (°C)	40 - 45
Iodine value (Wijs)	10 - 20
Mean molecular weight	330
Proportion of alcohols (%)	50 - 45
Alcohols: Melting point (°C)	55 - 65
Iodine value (Wijs)	40 - 50
Mean molecular weight	370

Table 3.2 Some physical and chemical data for wool wax [1].

3.2.1.1 Esters

Little is known about the way in which wool wax acids and alcohols are assembled into esters. Buisine in 1887 suggested that ceryl cerotate is present in wool wax [9], but Truter argues that there is no real evidence to support this [1].

Tiedt and Truter using an 85 stage systematic fractional crystallisation process managed to identify three wool wax esters from wool grease [10]: cholesteryl 24-methylhexacosanoate, cholesteryl 26-methyloctacosanoate and cholesteryl 28-methyltriacontanoate. A di-ester of a hydroxy-acid was also found but its components were not fully identified.

Crabtree and Truter using wool wax and thin-layer chromatography followed by gas-liquid chromatography [11] managed to identify 21 esters of cholesterol and 24 esters of lanosterol, together with 26 monocarboxylic acids which occur as units in α,β -diesters. Subsequently, Sydykov *et al.* using thin-layer chromatography followed by two rounds of column chromatography identified cholesterol isononatriacontanoate as present in wool wax [12].

It has been argued that wool wax esters must contain specific combinations of acids and alcohols [1], and it has been calculated that the wax must contain at least 8000 mono- and diesters, in addition to polyesters. This contrasts with only about 50 esters, as described above, having been identified so far.

However, it is possible to make some generalisations about the constitution of the esters [11]. Monoesters are derived by combination of monocarboxylic acids with polycyclic monols, namely, cholesterol and lanosterol and its congeners with only a trace of monoester being derived by combination of simple acid with long-chain alcohol. In contrast, the alcoholic components of diesters of α -hydroxy acids do not include polycyclic alcohols, but are exclusively long-chain alcohols. Each type of ester has its own particular quantitative pattern of acids, which differ in the distribution of both the isomers and the molecular weights.

Much more is known of the constituent acids and alcohols of wool wax. Saponification of the wax with alcoholic alkali yields the saponifiable portion containing the wool wax acids and the unsaponifiable portion containing the wool wax alcohols and hydrocarbons [2,5,13].

3.2.1.2 Acids

The isolation and identification of wool wax acids has been reviewed [1,5,13,14]. As the fractionation techniques used have improved, the number of wool wax acids identified have increased (Table 3.3).

Year	Number of Various Acids	Reference
1945	32	15
1960	69	16
1962	76	17
1967	about 90	18
1973/4	138 (plus 42 unsaturated acids)	19,20

Table 3.3 Number of various acids identified in wool wax acids [14].

Wool wax acids consist predominantly of alkanoic, α -hydroxy and ω -hydroxy acids. Each group contains normal, iso and anteiso series of various chain length, with practically all the acids being saturated [14].

The most recent complete study of the composition of wool wax acids was published during 1973-1974 by Fawaz *et al.* [19,20], using ‘pharmaceutical lanolin’ as the starting material. These results are shown in Table 3.4. However, as shown in Section 3.2.1, the composition of wool wax samples can vary, and so can the analytical techniques used. Hence a more representative, average composition of wool wax acids, based on the data of Downing *et al.* [16], Truter [17] and Fawaz *et al.* [19,20] is shown in Table 3.5. The acids of wool wax are indeed a complicated mixture.

Acids	Number of Acids ^a	Chain Length	Chain Length of Major Fractions	Wool Wax Acids %
Normal acids	27	C ₈ -C ₃₈	C ₁₃ , C ₁₆ , C ₂₄ , C ₂₆	22.1 ^b
Iso acids	17	C ₈ -C ₄₀	C ₁₄ , C ₁₆ , C ₁₈ , C ₂₀ , C ₂₆	22.1
Anteiso acids	18	C ₇ -C ₄₁	C ₁₄ , C ₁₉ , C ₂₁ , C ₂₅ , C ₂₇	26.3
α-Hydroxy acids normal	23	C ₁₀ -C ₃₂	C ₁₆	21.8
α-Hydroxy acids iso	12	C ₁₂ -C ₃₄	C ₁₈ , C ₂₄	4.5
α-Hydroxy acids anteiso	12	C ₁₁ -C ₃₃	C ₂₃ , C ₂₅	0.8
ω-Hydroxy acids normal	14	C ₂₂ -C ₃₆	C ₃₀ , C ₃₂	3.0
ω-Hydroxy acids iso	8	C ₂₂ -C ₃₆	C ₃₀ , C ₃₂	0.8
ω-Hydroxy acids anteiso	7	C ₂₃ -C ₃₅	C ₂₅ , C ₃₁	1.3
Polyhydroxy acids			No details	4.7
Total	138			
Unsaturated acids	42		C ₁₆ , C ₁₈	2.1
Total				109.5

^a Includes acids in trace amounts
^b Correct figure is most likely 12.1%

Table 3.4 Summary of the composition of wool wax acids [21].

Acids	Chain Length ^a	% of Wool Wax Acids
Normal acids	C ₈ -C ₃₈	10
Iso acids	C ₈ -C ₄₀	22
Anteiso acids	C ₇ -C ₄₁	28
Normal α-hydroxy acids	C ₁₀ -C ₃₂	17
Iso α-hydroxy acids	C ₁₂ -C ₃₄	9
Anteiso α-hydroxy acids	C ₁₁ -C ₃₃	3
Normal ω-hydroxy acids	C ₂₂ -C ₃₆	3
Iso ω-hydroxy acids	C ₂₂ -C ₃₆	0.5
Anteiso ω-hydroxy acids	C ₂₃ -C ₃₅	1
Polyhydroxy acids		4.5 ^b
Unsaturated acids		2 ^b
Total		100%

^a [19,20]
^b Tentative data

Table 3.5 Summary of the average composition of wool wax acids (1960-1974) [14].

3.2.1.3 Alcohols

The isolation and identification of wool wax alcohols has been reviewed [1,5,13,22]. As with the wool wax acids the number of alcohols identified has increased as analytical techniques have improved. Thus the number of components of the aliphatic alcohols reported between 1955 and 1974 rose from 17 to 69 [22].

The unsaponifiable portion of wool wax consists of aliphatic monoalcohols and alkane 1,2-diols, cholesterol, triterpene alcohols, and small amounts of hydrocarbons (see Section 3.2.1.4) and auto-oxidation products (see Section 3.2.2) [22]. The monoalcohols and the diols consist of normal, iso and anteiso series.

The most recent complete study of the composition of the unsaponifiable portion of wool wax was published in 1974 by Fawaz *et al.* [23,24], using lanolin. These results are shown in Table 3.6. The same restrictions apply to the unsaponifiable portion as to the wool wax acids: the composition can vary, as can the analytical techniques used. Accordingly, an average composition of the unsaponifiable portion of wool wax, based on the results of Murray and Schoenfeld [25], Horn [26], Downing *et al.* [16], and Fawaz *et al.* [23,24], is shown in Table 3.7. Cholesterol and the triterpene alcohols are the major components of wool wax alcohols. They are present in about equal amounts and together they represent about 72% of the total unsaponifiable material. The total aliphatic alcohols represent about 22%.

Components	Number of Identified Alcohols	Chain Length	Chain Length of Major Fractions	% of Unsaponifiable ^a
Monoalcohols, normal	16	C ₁₄ -C ₃₄	C ₂₄ , C ₂₆	1.6
Monoalcohols, iso	11	C ₁₄ -C ₃₆	C ₂₀ , C ₂₂ , C ₂₆	6.5
Monoalcohols, anteiso	11	C ₁₇ -C ₃₅	C ₂₁ , C ₂₅ , C ₂₇	9.0
1,2 diols, normal	14	C ₁₂ -C ₂₅	C ₁₆ , C ₁₈ , C ₂₀	0.4
1,2 diols, iso	9	C ₁₄ -C ₃₀	C ₁₈ , C ₂₀ , C ₂₂ , C ₂₄	5.9
1,2 diols, anteiso	8	C ₁₅ -C ₂₉	C ₂₁ , C ₂₃	2.4
Cholesterol				38
Dihydrocholesterol				trace
Cholesta-3,5-diene-7-one				3.6
Lanosterol				14.8
Dihydrolanosterol				10.3
3-hydroxylanosta-8-ene-7-one				1.6
Hydrocarbons				{ 5.9
Undetermined				
Total				100.0

^a Calculated from [23,24].

Table 3.6 Composition of the unsaponifiable portion of wool wax [22].

Components	Chain Length ^a	% of Unsaponifiable
Normal mono-alcohols	C ₁₄ -C ₃₄	2
Iso mono-alcohols	C ₁₄ -C ₃₆	{ 13 (1:1)
Anteiso mono-alcohols	C ₁₇ -C ₃₅	
Normal alkane 1,2-diols	C ₁₂ -C ₂₅	1
Iso alkane 1,2-diols	C ₁₄ -C ₂₀	{ 6 (1:0.5)
Anteiso alkane 1,2-diols	C ₁₅ -C ₂₉	
Cholesterol		34
Lanosterol		{ 38
Dihydrolanosterol		
Hydrocarbons		1
Auto-oxidation products and undetermined		5
Total		100

^a [23,24]

Table 3.7 Summary of the average composition of the unsaponifiable portion of wool wax (1955-1974) [22].

3.2.1.4 Hydrocarbons

The isolation and identification of wool wax hydrocarbons has been reviewed [13,27]. They represent about 0.5% of wool wax and consist of a large number of normal and branched homologues. This shows a structural similarity between the hydrocarbons and the wool wax acids or wool wax aliphatic alcohols: all three materials contain normal, iso and anteiso series. The wool wax hydrocarbons also contain highly branched alkanes as well as cycloalkanes [27].

Downing *et al.* [16] were the first investigators to gas chromatograph the wool wax hydrocarbons present in the unsaponifiable material. Table 3.8 lists the number and chain length of the normal, iso- and anteiso-hydrocarbons which they found. The isohydrocarbons are homologues of even carbon numbers whereas the anteiso-hydrocarbons are homologues of odd carbon numbers.

Hydrocarbons	Number	Chain Length
Normal hydrocarbons	21	C ₁₄ -C ₃₂ C ₁₃ -C ₃₃
Isohydrocarbons	10	C ₁₄ -C ₃₂
Anteishydrocarbons	8	C ₁₅ -C ₂₉
Total	39	

Table 3.8 Naturally occurring hydrocarbons in wool wax unsaponifiable [16].

The most recent complete study of wool wax hydrocarbons was published in 1970 by Fawaz *et al.* [28], using pharmaceutical-grade lanolin. The hydrocarbon fraction was found to represent 0.57% of the original lanolin. The hydrocarbons were separated into normal-, 'slightly branched' and 'highly branched' fractions, which were investigated using gas chromatography and mass spectrometry.

The normal hydrocarbons (16% of the total hydrocarbons) were found to have a chain length of C₁₃ to C₄₂, with trace amounts of C₉ to C₁₂ and C₄₃ to C₅₀. The major fractions consisted of C₁₉ to C₃₁. The normal hydrocarbons were observed to consist of two distinct families : C₁₃ to C₂₄ and C₂₅ to C₄₂.

The 'slightly branched' hydrocarbons (7% of the total) contain methyl-, cyclohexyl- and phenyl alkanes. The individual members vary from C_{16} to C_{48} .

The 'highly branched' hydrocarbons (77% of the total) vary from C_{16} to C_{45} . They consist of paraffinic hydrocarbons (similar to pristane), cyclohexyl- and phenyl alkanes.

3.2.1.5 Other Components

Most of the other components of wool wax are minor constituents only. Drummond and Baker [29] reported that wool wax contains small amounts of phosphorus and nitrogen, and suggested that wool wax might contain phospholipids. However, Morris and Truter [30] later showed that wool wax does not contain phospholipids but does contain traces of polypeptides and inorganic phosphate. Manganese has also been found in wool wax [31].

Arsenic, sulphur, iron, calcium and silicon have all been detected in wool wax [1] and nonionic surfactants can be detected in wool grease [32] but the presence of these is only in the form of impurities arising from, for example, dipping and dirt.

Even after all the research described previously wool wax still contains 3-9% of indeterminable substances [13]. The amount unidentified has decreased over time as the analytical techniques used have improved. Part of the unidentified components are auto-oxidation products (see Table 3.7). Horn [26] found that the proportion of unidentified material in the unsaponifiable matter was 53.5% in 'tip' wax compared with only 3.0% in 'base' wax. Hence, it appears that detailed knowledge of wool wax components is confined to unoxidised wax. The auto-oxidation of wool wax is considered in the next section.

3.2.2 Weathering

Lifschütz in 1924 was the first to show that the amount of wool wax and its composition vary even within the individual staple [33]. He found that the composition of the wax obtained from the tippy portion of the staple to be very different from that of the wool wax taken from the wool nearer the roots (Table 3.9). The surface wax nearer the tips contains a much larger proportion of fatty acids; it also contains oxycholesterol in place of the ischolesterol present in the wax nearer the roots. These differences in chemical composition are due to hydrolysis and oxidation brought about by the combined influences of moisture, light and air.

Weathering alters the physical and chemical properties of wool wax. Many studies have observed the effect of natural weathering, although a few have also been carried out using artificial weathering, including a number on wool wax alcohols.

Property Measured	South American Crossbred Wool		Montevideo Merino Wool		Australian Merino Wool	
	Roots	Tips	Roots	Tips	Roots	Tips
Wax content (%)	9.20	4.30	22.60	19.50	24.27	14.21
Acid number of extracted wax	15.68	25.70	2.24	14.56	Almost neutral	17.92
Acid number after saponification	28.00	50.40	11.76	27.44	7.28	28.00
Free cholesterol	Traces	0	Traces	Traces	Large amount	Traces
Free ischolesterol	Large amount	0	Large amount	Traces	Large amount	Traces
Free oxycholesterol	0	Large amount	0	Large amount	Traces	Large amount

Table 3.9 Variation in wool wax from root and tip parts [33].

Weathering - observed either by comparing tip wax and root wax or by the effect of artificial treatment - leads to an increase in water soluble substances [34,35], acid number [26,36-41], ester value [26,40-42], saponification value [26,40], carbonyl number [43], peroxide number [34,42], acetyl value [41], hydroxy value [38] and sensitivity to heat [38] as well as exhibiting a greater UV absorbance in the region 220-280 nm [38] and a greater infra-red absorption at $5.95\ \mu\text{m}$ ($1680\ \text{cm}^{-1}$) or $1670\ \text{cm}^{-1}$ corresponding to $\alpha\beta$ -unsaturated ketones [26,38,39,44].

However, weathering leads to a decrease in iodine value [36,38,39,42,45] (indicating that the number of double bonds is decreasing), the unsaponifiable/the amount of alcohols [26,38,40], emulsification value (of the alcohols) [46] and melting point (of the alcohols) [46] as well as causing a drop in the proportion of cholesterol and isocholesterol, which is paralleled by an increase in resinous residue [26,39,44].

Some of the results reported are however in conflict with each other. Bernhard and Dreker [47] found that UV irradiation of anhydrous lanolin brought about a rise in the amount of free sterols precipitated by digitonin whereas Muirhead *et al.* [41] found that passing oxygen through molten wool wax alcohols caused the amount of material precipitable with digitonin to decrease!

Weathering of wool wax on the fleece causes the wax to darken in colour [38,39,48,49] whereas oxidative bleaching or surface auto-oxidation of most types of wax cause a lightening in colour [46,49,50]. The difference can be accounted for by the presence of impurities, such as copper and iron [42], which influence and catalyse the oxidation of the wax on the fleece.

The weathering of wool wax has an effect on its recovery from wool scour liquors [38,51,52]. Unoxidised wax (arising from wax from the fibre root) is easily recovered, while oxidised wax (arising from wax from the fibre tip) is more difficult to recover, and is of lower quality commercially [38]. Attempting to raise the yield recovered results in more oxidised wax being recovered and so the quality of the wax deteriorates.

The chemical composition of oxidised/weathered wool wax is not as fully established as that of unoxidised wool wax (see Section 3.2.1). A number of constituents have been identified, namely: cholesta-3,5-diene-7-one [53]; 7-oxocholesterol, cholestane-3 β ,5 α ,6 β -triol, 3 β -hydroxylanost-8-ene-7-one and 3 β -hydroxylanost-8-ene-7,11-dione [54]; 7-oxocholesterol [7]; agnosterol, dihydroagnosterol and oxygenated derivatives of cholesterol and lanosterol [26]; unsaturated acids (amounting to 1.13% of the original wax) showing some reactions typical of steroids [55]; cholest-5-ene-3 β ,7 β -diol [56] as well as 3 β -hydroxylanost-8,24-diene-7-one and 3 β -hydroxylanost-7,11-dione [57].

3.2.3 Protecting Agent?

Lanolin is a well known corrosion inhibitor but the anti-weathering role of wool wax on the fibre is still under discussion.

3.2.3.1 In Industry

Lanolin, on its own or as part of a mixture, is a good rust preventive [e.g. 5,58-64]. Its property of oxidising in air to give a more resistant surface film plays an important part in its protective action [1].

The slightly tacky protective film behaves like a very viscous liquid so that the dust particles which are trapped by the tacky surface, will eventually sink into it. If the size of the particles exceeds the thickness of the protective film, then the particles will be in contact with both the metal and the atmosphere, and will be able to convey corrosive moisture to the metal surface [65]. Hence, the protective film of wool wax needs to exceed a critical minimum which is fixed by the type of corrosion likely to be encountered. The particle size of non-industrial atmospheric dust ranges from 0.25 to just over 2 μ m, so that adequate protection will not be provided by films thinner than 2 μ m (about 1.84g.m⁻²) [65]. Industrial dusts have even larger particle sizes, up to 4 μ m, so that a correspondingly heavier film of wool wax is required; conversely, if the atmosphere is dust-free, films as thin as 1 μ m will provide adequate protection [66].

3.2.3.2 In Nature

The anti-weathering role of wool wax, and the other constituents of the yolk, has been the subject of a number of studies. Kertess in 1919 [67] investigating the effect of atmospheric agencies upon wool found that lanolin proved to be a harmful addition, while Waentig in 1923 [68] found that wool poor in fat (washed) was more sensitive to UV light than that richer in fat. von Bergen in 1925 [69] appears to have been the first to attempt to correlate the degree of damage due to weathering with the lack of wax. However, rearranging von Bergen's data (Table 3.10) shows that there is no correlation. Additionally, the values for the single fleece he examined are so different from normal that they cannot be accepted as representative [1] but nevertheless von Bergen did show that the natural pigments and the dirt present in the fleece may help to protect it.

Sommer in 1927 [70] exposed samples of Melton cloth to natural weathering conditions for one year and found a small but unmistakable protective action by wool grease (see Table 3.11). He did not investigate the effect of suint.

A similar small protection against UV irradiation was observed by Hambrock and Wilken-Jorden in 1934 [71], using the Pauly-Rimington method. (This investigates the epicuticle and is very sensitive, becoming useless when the damage is at all extensive.) However, they were not convinced of the reality of the protection, because in a later experiment desuinted wool was found to be less damaged than both raw and clean wool.

Sample	Solubility Number (%)	Wax Content (%)
Belly staple	0.12	6.12
Back staple	0.12	1.62
Side staple	0.25	3.83
Belly tip	0.25	0.37
Side tip	0.75	0.90
Back tip	4.5	0.90

Table 3.10 Possible correlation between wool damage and wax content [69].

Conditions	Tensile Strength (arbitrary units)	Extension at Break (%)
Clean, without exposure	12.62	29.6
Raw wool grease (14.4%)	4.90	16.0
Olive oil (11.8%)	4.73	14.8
Wool grease (0.2%)	3.15	7.7
Clean, after exposure	2.80	7.7

Table 3.11 Physical properties of pure wool Melton after a year's weathering [70].

A more extensive investigation of the protective action of fleece constituents against damage by light from a mercury vapour lamp, was carried out by Truter and Woodford in 1955 [72]. Damage was measured by two methods, alkali solubility, and reduction in work to stretch. They were chosen as being indicative of the total behaviour of wool.

In the first group of experiments, variously treated bundles of Merino wool were irradiated in a Fadeometer (where the light source is a glass-shielded carbon arc). Later, samples of flannel made from Merino wool, were exposed to the light emitted by a Hanovia lamp. The results showed that there was some doubt as to whether the wax was able to afford any protection, but the protective action of suint was unmistakable (Table 3.12).

Coating	Alkali Solubility			
	Wool Fibres (at 65°C)		Flannel Samples (at 60°C)	
	Number of Observations	Mean ^a	Number of Observations	Mean ^a
None	3	18.6±1.0	5	22.0±0.8
Wax	3	16.8±1.5	10	20.6±0.7
Suint	3	14.8±0.2	5	15.0±0.2
Wax and Suint	3	16.6±0.3	5	15.3±0.3
Raw	3	10.0±0.6	-	
None, unexposed	3	10.4±0.1	3	6.9±0.1

^a The deviation stated is the root mean square.

Table 3.12 Alkali solubilities of variously treated wool samples after UV exposure [72].

In the second group of experiments, the reduction in work to stretch single Lincoln wool fibres was measured. Lincoln fibres had to be used because Merino wool is too weak and too crimped to give satisfactory results. This raises the question of the effect of the different amount of wax present on Merino and Lincoln fibres (Table 3.1). However, in considering the protection of the fibre, the thickness of the wax layer is the important factor. Calculations revealed that Lincoln wool has a wax layer of $1.5\mu\text{m}$ which is about two-thirds as thick as that on Merino wool ($2.0\text{-}2.3\mu\text{m}$).

The results (Table 3.13) show that a film of wax of the thickness occurring naturally on the fibre, is insufficient to protect it, but a heavier, artificially applied layer can. That the protective action is due to the exclusion of moisture and/or oxygen, and not to a decrease in the intensity of irradiation, was shown by placing clean fibres behind a quartz screen, part of which had been coated with a layer of wax $20\mu\text{m}$ thick. Both groups of fibres were damaged to the same extent (Table 3.13). That the natural wax layer is freely permeable to water vapour (and presumably to oxygen as well) is indicated by the observation that dried, waxed wool conditions to its normal moisture regain at about the same rate as does clean wool [1]. No measurements for the protective action of suint were possible by this method because the fibres must be stretched in water.

Fibre	Number of Observations	Reduction in Work (%) ^a
Exposed directly:		
Clean	9	21.5 ± 2.5
Natural (11% wax; $1.5\mu\text{m}$ layer)	6	22.5 ± 1.2
Waxed by hand (120% wax; $13\mu\text{m}$ layer)	9	11.8 ± 2.4
Exposed behind quartz shield which was:		
Clean	8	19.9 ± 3.0
Waxed ($20\mu\text{m}$ layer)	10	18.5 ± 3.5

^a The deviation stated is the root mean square.

Table 3.13 Reduction in the work done in stretching single fibres 30% in water [72].

The protective action of suint found could not be attributed to the absorption of light as the screening effect of wool wax and suint is approximately the same [1]. Whether suint protection is an effect of major importance in the fleece is questionable because only the primary fibres are equipped with accessory sudoriferous glands (Section 3.1) (which in the Merino fleece comprise about one twenty-fifth of the total). Secondary fibres will not therefore be protected unless the suint is able to mark off on to them.

Le Roux in 1958 [45] investigated factors which damage or protect wool during growth. He found that the (high) density of the fleece and, to a lesser extent, a fluid and adhesive wool wax are two of the factors which restrict damage in the tip portion of wool staples. A fluid and adhesive wool wax allows the fibres to pack tightly against each other in the wool staples of dense fleeces and so protect against degradation.

He also found that penetration of dirt into the wool staple promotes damage to the wool and that wool samples with abundant yolk were not protected more effectively than those containing less yolk.

Louw *et al.* in 1963 [73] studied the influence of artificial weathering (sunlight behind window glass) on Merino wool. By observing the extremely rapid destruction of cystine during the initial period of irradiation and comparing this with the normal weathering on the sheep's back they illustrated the disastrous effect of opening up the staple so as to expose a larger total of fibre surface to irradiation.

They found it noticeable that raw wools were less seriously effected by irradiation than their cleaned counterparts (in their weathering procedure they had spread out fibre sections evenly, giving a density of about a gram of clean wool per 10 cm², before irradiating). However they decided that grease and suint did not afford protection *per se* to the single wool fibre, but served to restrict the penetration of weathering factors, especially light and sand, into the fleece on the sheep's back by virtue of their cementing action.

Mezentsev in 1970 [74] investigated the physiological role of the luminescence of wool fat. He found that wool fat acts as a biological transformer transforming part of the short wavelength radiation (UV and violet), which has an active biological effect, into long wavelength radiation. This altered the spectral distribution of the radiation reaching the wool which caused the wool to become warmer. Hence he implicitly comments that wool fat decreases the amount of harmful radiation reaching the wool fibre.

Zhu *et al.* in 1981 [75] studied the relationship between wool wax content and wool anti-weathering property. They used wool samples which naturally had different amounts of wax on them (30, 25, 18, 15 and 10%) and artificially weathered using a Xenotest 150. The anti-weathering ability of wool decreased with increasing wool wax content with a demarcation around 18% wool wax content. After weathering, the physical and chemical properties of wool fibre changed to a greater degree with wax content below the demarcation value than above it.

Zhao *et al.* in 1982 [76] investigated the relationship between the colour of wool wax and the anti-weathering property of wool fibres. They used wool samples which naturally had different amount of wax on them (37.20, 22.10, 21.37, 12.83 and 9.60%) and artificially weathered using a Xenotest 150. They found no correlation between the colour of the wax and the anti-weathering properties of the fibre. However, they did find that the amount of wax was related to resistance to weathering, with a wax content of 37.2% having good resistance whereas with wax contents of 12.83% and 9.60% a worse resistance was obtained.

It is appropriate here to report a few related investigations. McCarthy [77] has proposed the use of lanolin derivatives in sunscreen preparations. He admits however, that lanolin oil itself has the properties of a limited UV erythral absorber (290-320 nm) which agrees with Truter and Woodford [72] that wool wax does not protect the fibre by the reduction in intensity of irradiation that it causes.

Tomlinson and Rich [78] found that tobacco leaves were made more resistant to ozone injury by applying an emulsion of lanolin.

Hay and Mills [79] investigated the migration of sebum and suint components along wool fibres of Merino sheep. They found that isotopically labelled wool wax is carried passively along the fibre as a band of growth of the wool, and that it does not flow along the fibre to any large extent during experiments of several months, supporting the view that the function of the sebum is to protect the wool fibre and the condition of the coat. Also, it seemed unlikely that wax lost from the fleece during exposure to rainfall is replenished with sebum freshly secreted onto the surface, as sebum does not migrate.

Isotopically labelled suint components were also found to be carried along passively by wool growth and it was regarded as unlikely that wax is transported to any extent by suint in the wool. Some spreading of radioactive wax and suint bands was noted and ascribed to possible diffusion and mechanical disturbance of the wool.

To briefly conclude, it can be said that suint and the density of the fleece protect the wool fibre. Wool wax at high concentrations protect the fibre (by exclusion of oxygen and/or water rather than by absorption of light) but at low concentrations the situation is unclear. Dirt promotes damage.

3.2.4 Luminescence

Little has been reported about the luminescence of wool wax. Croner in 1926 [80] appears to have been the first to describe the fluorescence of lanolin. Using UV irradiation he found that lanolin emitted a weak bluish fluorescence. However, Hirst in 1927 [81] while describing the fluorescence of some wools found that clipped Merino greasy gave a yellow colour with bluish-white tips but that the 'extracted fats, & c.' are themselves non-fluorescent.

van Raalte in 1928 [82] investigated the luminescence of a number of oils and fats including 'sheep fat'. He observed that most, including sheep fat, did not luminesce in the crude state. Treatment with sulphuric acid or fuller's earth induced luminescence while decolorisation with norite had no effect. It was decided that a substance inhibiting luminescence was removed during these refining processes. The substance was assumed to be a sterol as it could be precipitated by digitonin. In many respects the substance gave reactions attributed to vitamins. van Raalte also left some fuller's earth refined sheep fat in a test tube in sunlight and after several weeks did not observe any luminescence.

Carrière in 1928 [83] disagreed with van Raalte's statement that only refined oils are fluorescent. Carrière said that all oils and fats are fluorescent, with some oils showing a greater absorption of ultraviolet light than others. He attributed van Raalte's results to using an incorrect observation technique.

It was not until Mezentsev in 1970 [74] that the next account of the luminescence of 'wool fat' occurred. By looking at sheep wool samples before and after fat removal he observed a strong depression of the luminescence after fat removal suggesting that the fat is responsible for the luminescence. The proteins in the wool showed no luminescence (which is very strange when one considers the extensive reports of the luminescence of wool in Section 2.5!)

As already implicitly stated not all the incident irradiation reached the wool fibre, some was absorbed and re-emitted as luminescence. Thus, the intensity and radiation spectrum reaching the wool is essentially different from the incident irradiation. A dispersion analysis showed that the luminescence depends on fat colour and not on breed of sheep. Out of the wool fats studied (yellow-red, yellow, light yellow, white and white-green) the highest luminescence was observed in the yellow-red and yellow fats.

The present work endeavours to increase the knowledge and understanding of, the nature of the chemical species responsible for, and the effect of light irradiation upon, the fluorescence of wool wax.

3.3 SUINT

Suint is usually defined as being that part of the raw fleece which is soluble in cold water [1]. This definition is entirely arbitrary, but it is useful from a practical standpoint. The aqueous extract will include a number of substances which cannot be regarded as true physiological products. For example, photochemical degradation of the fibre while it is still on the sheep's back, gives rise to water-soluble products containing nitrogen and sulphur. Other photochemical reactions may change the constitution of the glandular secretions.

Another factor of importance is the pH of the wool involved, as wool fibres are gradually degraded by the action of aqueous solutions at a rate which increases rapidly with rise in pH and temperature [1]. Even distilled water at 50°C has an appreciable effect [84] and will extract substances which are more properly regarded as part of the fibre. Other complications may be introduced by contamination of the fleece or the dried products of urine, by the presence of water-soluble vegetable and mineral matter, by chemical interaction between the secretions from the two sets of glands (sudoriferous and sebaceous), and by the growth of bacteria in the fleece.

Whether the suint is obtained from the fleece before or after the wax is removed makes a difference. The yolk contains a small fraction which is soluble in both water and fat solvents [85-87]. This does not seem to have been appreciated by early investigators.

The variability of the amount of wax in the wool of different breeds is accompanied by a similar variation in the amount of suint, which ranges from 2 to 15% [3]. The suint content of greasy wool is independent of wool quality (see Table 3.1).

Given the above considerations it is apparent that the results of analyses of suint may be at considerable variance with one another.

3.3.1 Nature and Composition

If raw wool is gently agitated in cold water and then removed, the resultant liquor is opaque, and appears to be a sol or an emulsion [1]. It can be filtered or centrifuged to give a clear solution, which is reddish-brown in colour, the depth of shade varying with the concentration. It has an unpleasant sheep-like odour, froths on shaking, and behaves very much like a solution of ordinary soap, although the pH is lower (usually about 8). It can be evaporated down to a thick, syrupy, hygroscopic concentrate. As a result of bacterial activity a clear solution becomes cloudy after standing for several hours. The ultraviolet spectrum, however, remains much the same.

Suint was first examined as long ago as 1802 by Vauquelin [88,89]. His work and later work up to the 1950's has been reviewed [1,86,90] but can be ignored as no experimental measurements have been adduced. A number of 'recent' studies have been carried out [86,91-101], of which the most thorough are by Deane and Truter [94] and by Howitt and Preston [86].

Deane and Truter [94] fractionated suint from Australian Merino wool by means of ion-exchange resins into four main fractions:

1. Inorganic cations 44% (Farnworth [93] found potassium (26.4%), sodium (1.3%), calcium (1.3%), magnesium (0.3%), aluminium (0.2%) and iron (0.04%), with the mean potassium content of greasy fleece being approximately 1.5%).
2. Organic acids and anions 36% including:
 - a) steam-volatile organic acids (2%), namely acetic (1.1%), propionic (0.2%), n-butyric (0.3%) and n-valeric (0.2%).
 - b) acids not volatile in steam and also insoluble in ether (18%), are dibasic, ranging from oxalic to pimelic but excluding malonic acid. Succinic acid (10%) and glutaric acid (1%) were found.
 - c) possibly keto-acids.
 - d) no steroids could be detected.

3. Weak bases 4%.
4. Ampholytes 3% (Earlier work by Deane and Truter [91] had shown that this fraction contained at least 11 peptides each of which contained between 8 and 13 different amino acids. Later work by Fraser and Truter [95] found that one-quarter of the nitrogen content of suint could be attributed to the α -amino groups of peptides, together with small amounts of glutamic and aspartic acids, isoleucine and taurine. They also found that hydrolysis of the peptides gave 20 identifiable amino-acids).

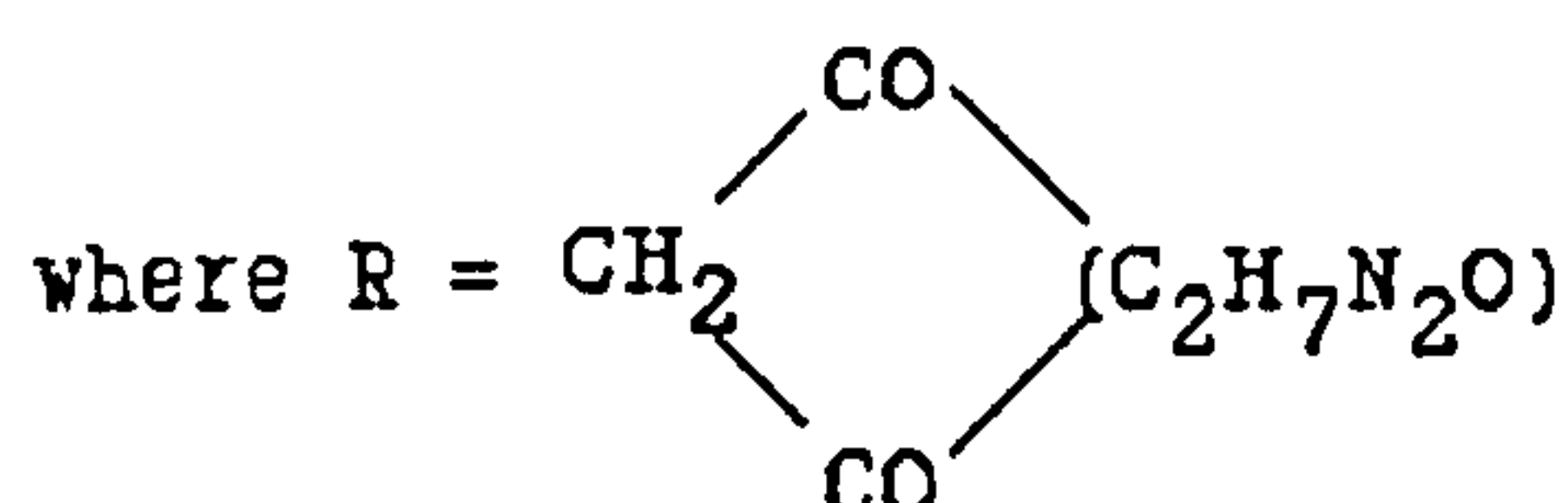
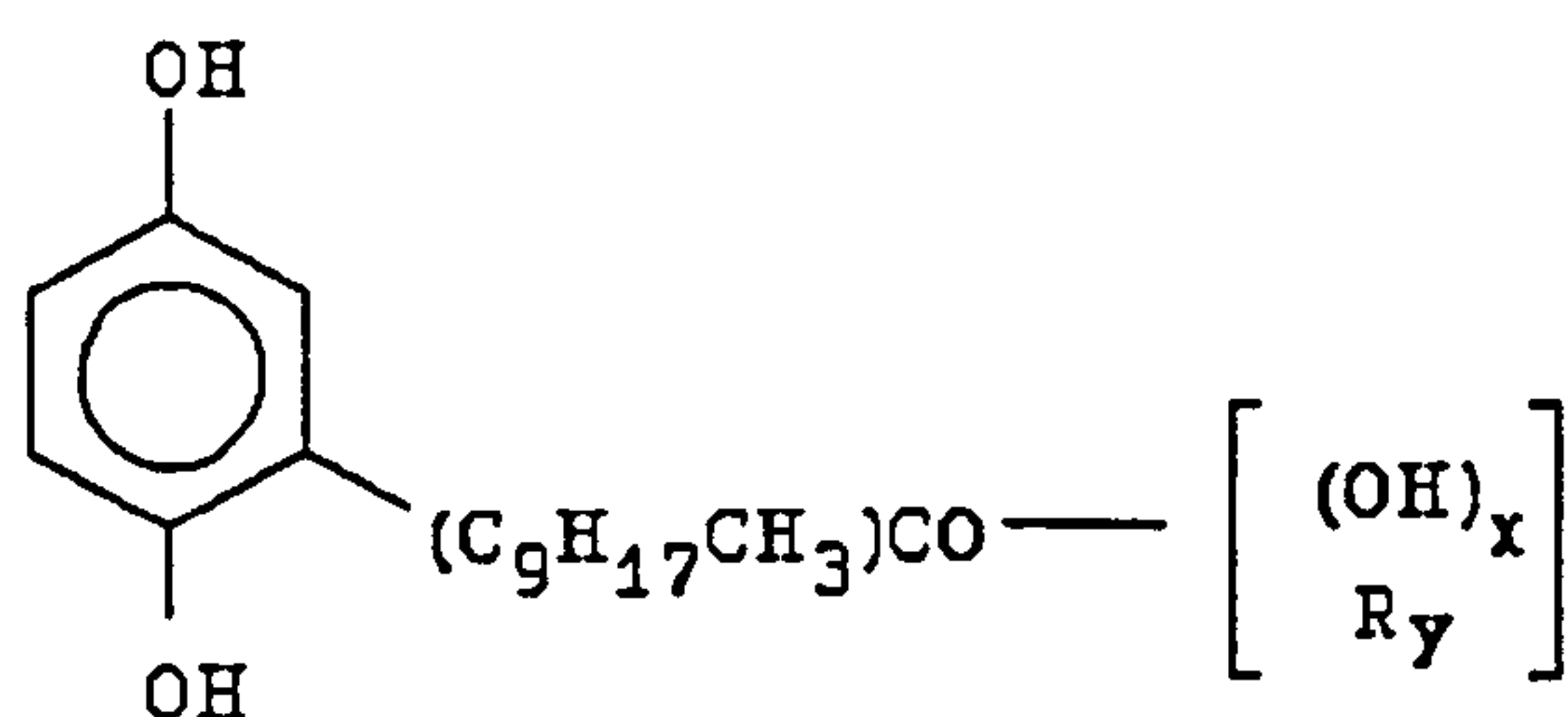
They attributed the large incomplete recovery to the presence of the ammonium ion and water-insoluble fatty acids which were originally present as water-soluble potassium salts.

The major part of work by Howitt and Preston [86] was carried out on the acidic portion of suint, separated by the use of ion-exchange columns. They found volatile acids, higher fatty acids, dicarboxylic acids, hydroxy- and keto-acids, aldehydes, sugar acids, phosphorylated sugars, polypeptide material, and urea. They could not find uric acid, creatine or creatinine. In the ash of suint the cations sodium, potassium, aluminium, iron, copper, magnesium and calcium were detected together with the anions chloride, sulphate, phosphate and silicate.

As well as the preceding components in suint there is also colouring matter present. The natural yellowness of wool, including the involvement of suint, has been reviewed [102,103]. Some of the reported pigments are as follows:

Rimington and Stewart [104] attributed 'golden' coloration to the presence of a pigment 'lanaurin'. They did not come to any definite conclusion regarding its chemical nature but suggested it was a tetrapyrrole derivative of the bile-pigment type with a formula $C_{33}H_{36}O_{10}N_4$.

Fraser and Truter [105] investigated the colouring matter from both fresh and aged suint from normal sheep but did not find any evidence for the presence of pyrrolic pigments. They found the fundamental molecular unit to be methyl 10-(2,5-dihydroxyphenyl)-decanoic acid, and this was associated, usually non-stoichiometrically, with a nitrogenous β -diketone:



and where $x+y=1$, the actual values depending on the history of the sample. Ageing of the suint caused the phenolic portion of the coloured molecule to polymerise.

Chipalkatti *et al.* [106], supported by Kenkare *et al.* [107,108], have shown that (unidentified) pigments present in the suint are responsible for canary colouration in Indian raw wools. The pigments enter the fibre when the grease content is low as the grease protects the fibres from the suint. Kenkare *et al.* [108] thought that the pigments should logically be found in the wool wax as well, which is what they found.

Some of the more readily noticeable colours in raw wool can be assigned to pigments produced as a consequence of the activity of microbes or fungus [109-117]. An infrequently observed black discolouration of the tip of wool staples resembling tar contamination has been attributed to the fungus *Peyronellaea glomerata* [110,111]. More usually however, the colour reported is blue, green, brown, apricot, purple, pink or red [114-118].

The occurrence of stains in wool arising from *Pseudomonas aeruginosa* has been reported [115-119]. The pigment produced is pyocyanin which is a green base. Its salts are red and its primary oxidation product, oxyphenazine, is yellow [117]. The pigments actually produced by *Pseudomonas aeruginosa* depend on for example the pH and the age of the sample [115,116]. Fraser and Mulcock [115] isolated four pigments from a chromogenic strain of *Pseudomonas aeruginosa* which had been obtained from the fleece of Corriedale sheep; a green fluorescent water soluble pigment; a blue fluorescent, chloroform and water soluble pigment; a blue, chloroform and water soluble pyocyanine with indicator properties; and a yellow, chloroform and water soluble hemi-pyocyanine with indicator properties.

Pseudomonas indigofera has also been identified as producing a blue pigment [118,120].

3.3.2 Protecting Agent?

The anti-weathering role of suint is considered in Section 3.2.3.2, along with the role of the other constituents of the yolk.

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CHAPTER 4

BACKGROUND EXPERIMENTAL

4.1 PREFACE

This chapter provides the explanation of the nomenclature used to describe the common materials and techniques used in this thesis, as well as describing the common techniques and instrumentation. It does not seek to be exhaustive but to provide the background information to the experimental sections in the remaining chapters, where some investigations differ only subtly from one another.

4.2 MATERIALS/CHEMICALS

All materials and chemicals were used as supplied, unless stated otherwise.

4.2.1 Wool

All samples were stored in the dark, unless stated otherwise, to prevent photo-oxidation.

4.2.1.1 Wool Fabric

The wool fabric employed was a botany serge (2/2 twill of weight 200gm⁻²) supplied by Parkland.

4.2.1.2 Wool Tops

Two wool tops were employed, which consisted of:

1. 'BA tops'. A Buenos Aires Lincoln wool top of 46s quality.
2. 'DWI tops'. A Merino wool top having a diameter of 21.0 μm , provided by Dr K. Schäfer of DWI. The tops were cleaned using Cleaning Method 4 (see Section 4.3.1) before use.

4.2.1.3 Wool Fibres

A number of raw wools were employed for fibre studies, which consisted of:

1. 'Merino fleece 1'. A raw wool (ex. Heydemann Shaw), average diameter 21.5 μm .
2. 'Merino fleece 2'. A raw wool, average diameter 23.7 μm .
3. 'Merino fleece 3'. A raw wool, of 64s quality.
4. 'Sharlea fleece 1'. This was supplied by Dr D. Rivett, of CSIRO, and comes from sheep which have been specially bred and protected from most climatic exposure. The sheep are kept in open sided sheds, hand fed and have no direct exposure to sunlight. The wool is very white and has an average diameter of 17.0 μm .
5. 'Sharlea fleece 2'. This was also supplied by Dr D. Rivett, of CSIRO, and is similar to Sharlea fleece 1 except that the staples were the normal yellow colour of wool, indicating that the staples probably had some accidental exposure to sunlight. The wool has an average diameter of 17.4 μm .

4.2.2 Wool Wax/Wool Grease

One sample of wool wax was used. This was obtained from Merino fleece 1 by using Cleaning Method 1 (see Section 4.3.1.1). After cleaning, the solution was twice filtered before the solvent (tetrachloroethene, technical grade (BDH)) was evaporated under reduced pressure. The wool wax was still fairly dirty.

A number of wool greases were employed, which consisted of:

1. 'Croda wool grease'. This was supplied by Mr A. Safda, but originated at Croda Ltd.
2. 'Merino wool grease'. Heydemann-Shaw Centrigugal wool grease Merino's quality, supplied by Mr B. Robinson, IWS.

3. 'W1-W6'. Six New Zealand wool greases containing chlorophyll contamination, supplied by Dr D.A. Rankin, WRONZ. On receipt, the wool greases were homogenised, by melting, stirring and leaving to cool, before any analysis took place. The wool greases are described in Table 4.1. W1 was obtained from scouring a 'dag wool line', i.e. a wool blend containing a large amount of wool contaminated with dags (small balls or lumps of faeces-encrusted wool shorn from the crutches of sheep). W2, W4 and W6 are from the top of their respective drums, which had undergone appreciable oxidation, resulting in them being considerably lighter than their parent wool greases (W1, W3 and W5 respectively). The oxidised wool greases are considerably tackier and harder than their parent wool greases.

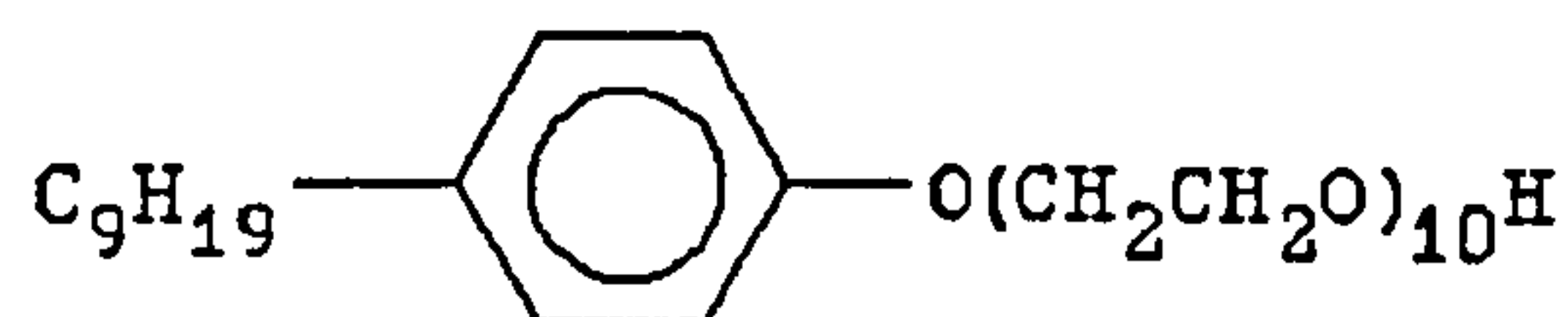
Code Name	Colour of Grease	Dr Rankin's Assessment of Chlorophyll Contamination	Sample Obtained from the:
W1 W2	dark brown greeny wax	} heavy	bulk of a small drum top of the same drum
W3 W4	light brown golden yellow wax	} lesser	bulk of a small drum top of the same drum
W5 W6	greeny wax waxy	} a typical New Zealand grease, of good colour and quality	bulk of a small drum top of the same drum

Table 4.1 Description of New Zealand wool greases.

4.2.3 Chemicals

All chemicals employed were laboratory reagent grade unless stated otherwise.

Some of the investigations used Lissapol N (ICI). This is a wetting agent and has the structure shown below [1]. It is necessary, because of the hydrophobic nature of wool, to enable aqueous solutions to penetrate the fibre. It also serves to achieve a level treatment (of, for example, dye) across the surface of the wool sample.



Lissapol N - 28% active

4.3 METHODS

4.3.1 Cleaning Methods

A number of cleaning methods were employed for cleaning fibres, which consisted of:

1. 'Cleaning Method 1'. Wool staples were cleaned by twice rinsing/shaking for 5 minutes in tetrachloroethene (BDH) at room temperature [2]. This was followed by damping with paper towels before leaving the wool staples to dry at room temperature in the dark. Initial cleaning used technical grade solvent, subsequently normal laboratory reagent grade was used. This cleaning method was only used for early investigations. An appreciation of the fluorescence quenching properties of chlorinated solvents through the heavy atom effect (see Section 1.4.4) and the likelihood of solvent penetration/retention (see Section 2.6.2) led to this method no longer being used.
2. 'Cleaning Method 2'. Wool staples were cleaned using a slightly modified version of the method of Leeder and Marshall [3] to ensure that the fibre surface was cleaned without removing any labile material from the interior of the wool. The solvents, t-butanol (2-methylpropan-2-ol) and n-heptane, were dried by twice fractionally distilling, the latter distillation over sodium.

Wool staples were dried under vacuum over phosphorus pentoxide (periodically changed) in a desiccator for 1 day and then extracted in the dark under strictly anhydrous conditions (involving dried solvent with the addition of molecular sieve and the bubbling through of nitrogen before standing) with t-butanol (30°C, 3 days), n-heptane (35°C, 1 day), and t-butanol (35°C, 1 day). (In between solvents the wool was damped with filter paper and left in a warm oven (~50°C) for 5 minutes). The wool was then washed in several changes of distilled water over 24 hours at room temperature in the dark, damped with filter paper, dried under vacuum over phosphorus pentoxide (periodically changed), given an ultrasonic treatment (Transistor/Ultrasonic T-21) under n-heptane (including molecular sieve) for 6 hours to remove any remaining dirt and skin debris, followed by air-drying at room temperature in the dark.

3. 'Cleaning Method 3'. Although Cleaning Method 2 works it is very laborious and not ideal for routine analytical analysis. Hence, another method designed to ensure that the surface of the wool fibre was cleaned without removing any labile material from the interior of the wool was used [4].

Wool staples were dried at room temperature by leaving them under vacuum over phosphorus pentoxide overnight (or longer) in the dark before soxhlet extracting the staples with anhydrous t-butanol for 2 hours [5], giving a quick rinse in n-pentane (Rathburn, hplc grade), and soaking overnight (or longer) in distilled water (1 litre) before drying. Initially this drying was performed by leaving the staples under vacuum over phosphorus pentoxide in the dark; subsequently this was carried out by air-drying at room temperature in the dark.

The t-butanol was dried by refluxing AnalaR grade t-butanol (BDH) (or distilled laboratory reagent grade) over sodium, before fractional distillation.

Initial soxhlet extractions were carried out using normal cellulose thimbles. These proved only partially effective as they retained the dirt from the wool staples. Extracting with no thimble also proved only partially effective as the wool fibres restricted the siphoning away of the dirt. Subsequently the wool staples were held in a stainless steel thimble [6] (designed and made at City University) which consisted of the sides having a 'honeycomb' structure and the end a wire gauze.

4. 'Cleaning Method 4'. Wool top was cleaned by rinsing in water before soxhlet extraction with dichloromethane for 4 hours, followed by rinsing and drying at ambient temperature.
5. 'Cleaning Method 5'. Wool staples were scoured (cleaned) using a laboratory size copy of an industrial scouring process [7]. Wool staples were washed using a sequence of 5 'tanks' (1ℓ beakers). The first contained 1g.ℓ⁻¹ Nekanil 910 (BASF, non-ionic detergent) at 55-60°C, the second contained 0.5g.ℓ⁻¹ Nekanil 910 at 55-60°C and the others contained only water at 50°C. Tap water was used throughout. The wool spent 3 minutes in each tank; being passed through a mangle between each tank such that the post-mangle weight was only 40% of the pre-mangle weight. The wool staples were dried in the open laboratory (i.e. received some light exposure). Trials indicated the possibility of felting so the wool staples were tied about one third of the way from the tip using thread, before cleaning. This was not totally successful.

4.3.2 Irradiation Methods

A number of irradiation methods were employed for irradiating samples, which consisted of:

1. 'Irradiation Method 1' (or 'light box'). The basic arrangement comprised placing the staples under an array of 3 x 18/20W fluorescent tubes in a 'light box' [1] for the appropriate length of time. For UV irradiation, tubes having a maximum emission at approximately 365 nm were used - either UV (Thorn, 20W) or Blacklight (Philips, 18W, incorporating an internal filter to reduce radiation in the visible spectrum). For blue light irradiation either Northlight (Thorn, 20W) or Northlight 55 (Philips, 20W) were used. The spectral outputs of these types of lamps are shown in Figure 4.1 (the intensities are not necessarily correct). The Northlight lamps emit a small amount of UV. To remove this a filter was used. Initially Perspex was used (6mm, ICI) but later laminated glass (6mm, P. Wigan, Glass & Glazing, 160 Liverpool Road, London) was used as this has a higher cut off point [10]. The transmission properties of these filters, together with a sample of normal glass (5mm), are shown in Figure 4.2.
2. 'Irradiation Method 2'. Fabric was irradiated with a helium cadmium laser, model 4240 NB (Liconix, Sunnyvale, USA), wavelength 442 nm, output power 50 mW. A lens was placed between the laser and the fabric such that the diameter of the beam impinging on the fabric was 5mm.
3. 'Irradiation Method 3'. Fabric was irradiated with a Spectra-Physics Inc. 2000 series (California, USA) Argon/Krypton/15W laser with the assistance of Mr R.A. Valser (City University). A lens was placed between the laser and the fabric such that the diameter of the beam impinging on the fabric was 2 cm. The blue line (488 nm, 7-8 W) and the UV line (351 nm, 1.3-1.5 W) were used. Where a fabric was irradiated wet the fabric had been previously dipped in aqueous Lissapol N and rinsed very thoroughly. The sample was also subsequently 'sprayed' periodically during irradiation (after 0.5 hour for 1 hour irradiations, and every hour for 4 hour irradiations). After irradiation the sample was left to dry at room temperature in the dark.

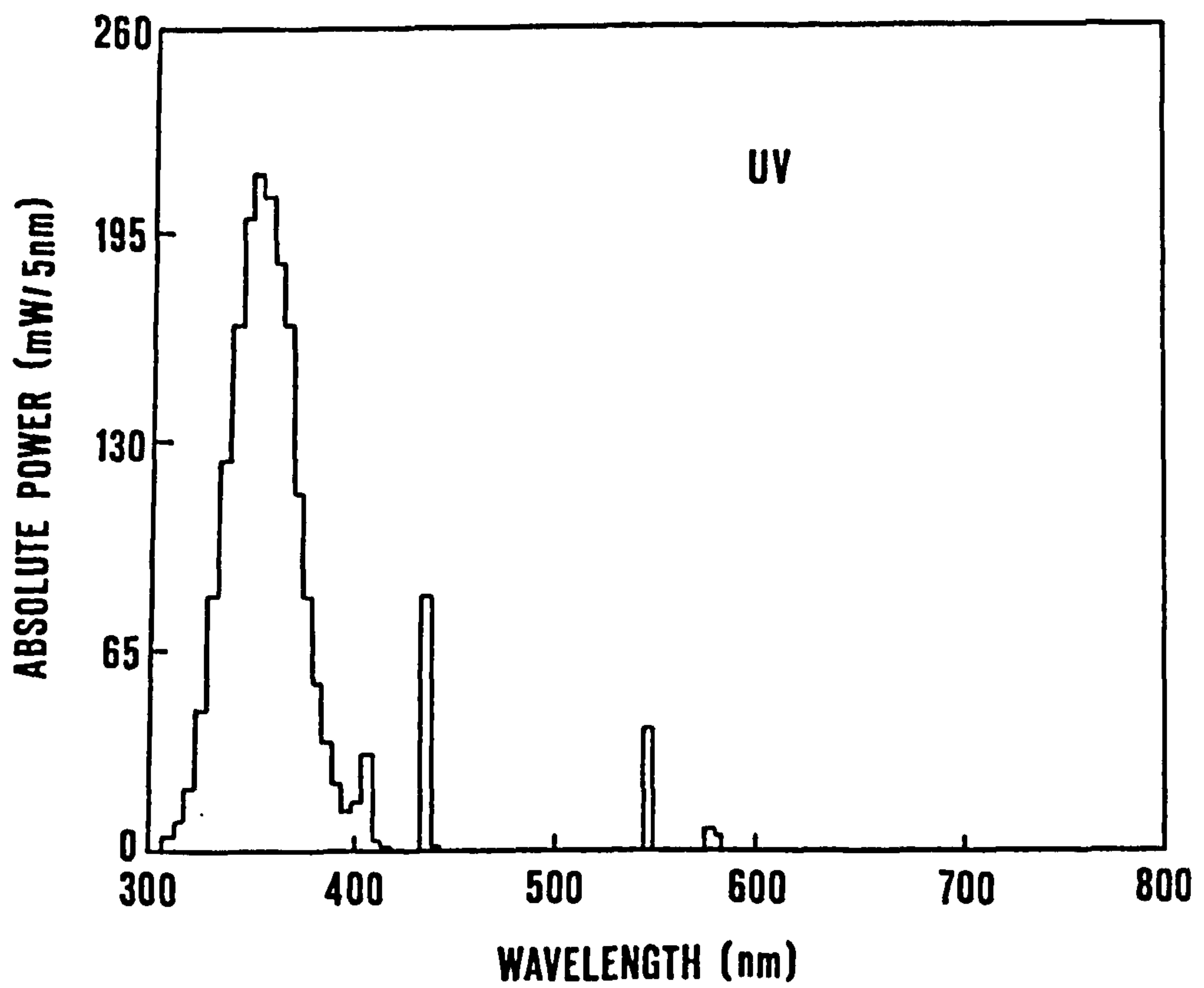
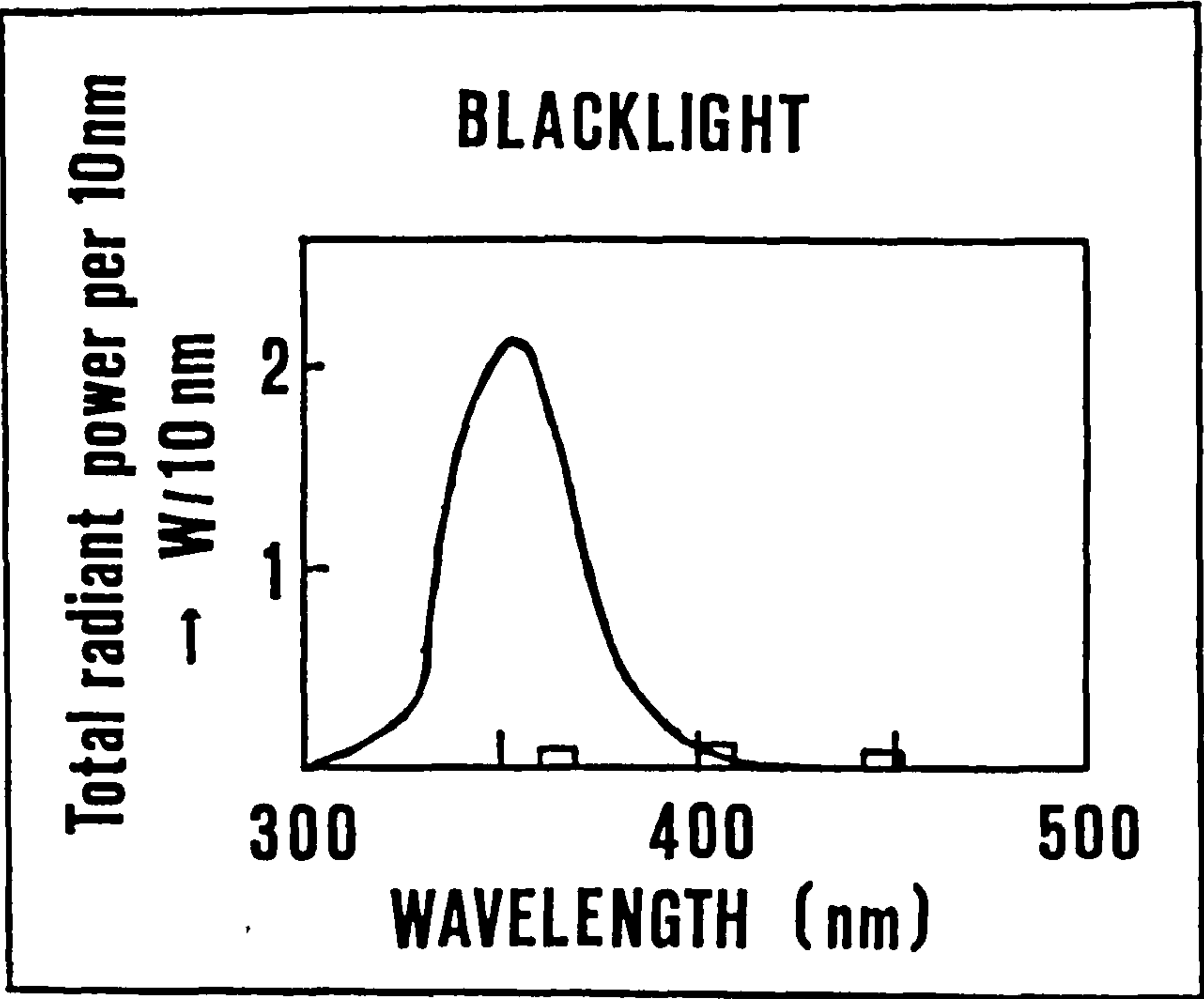


Figure 4.1 Spectral emissions of various fluorescent tubes (redrawn) [8,9].

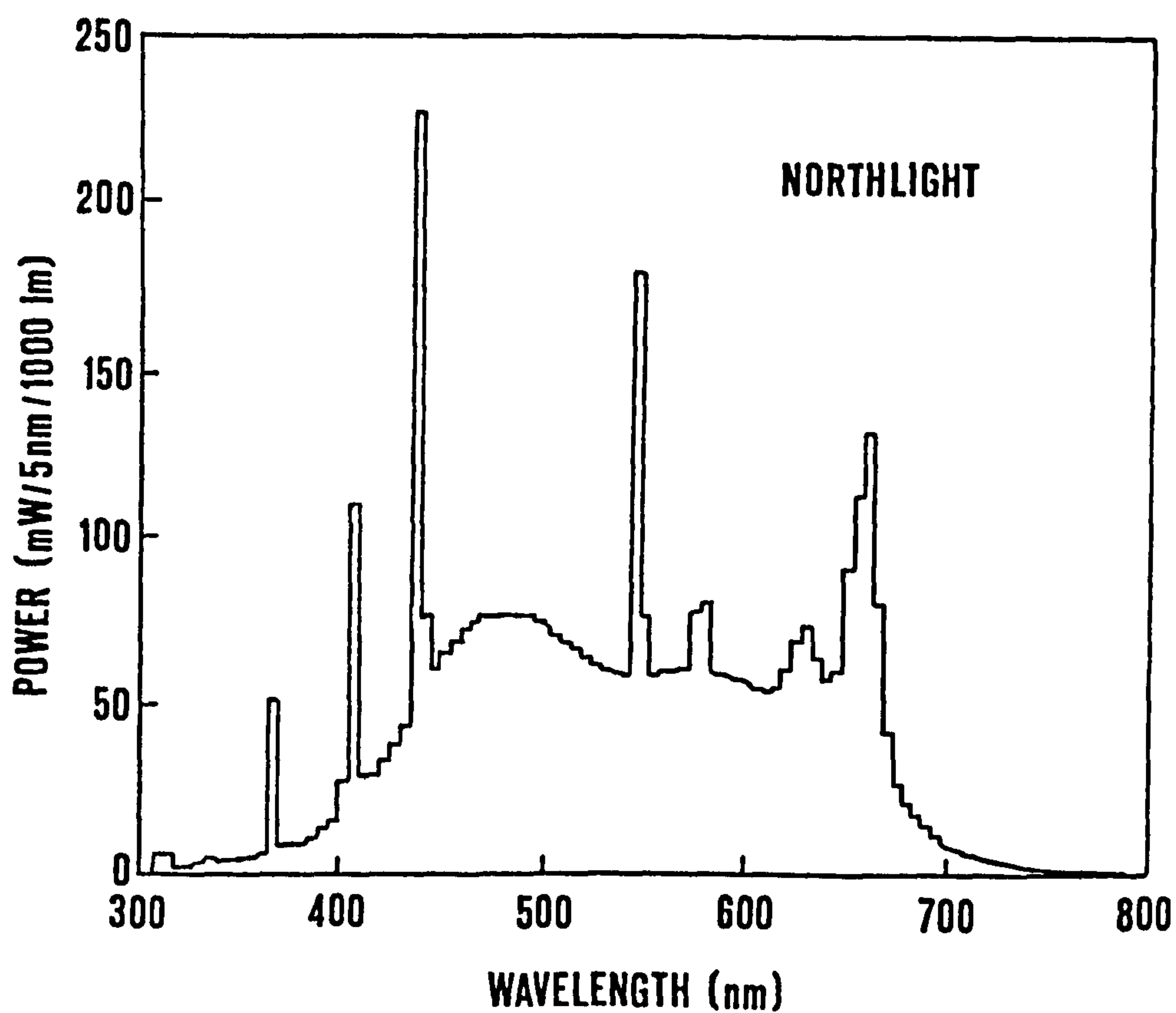
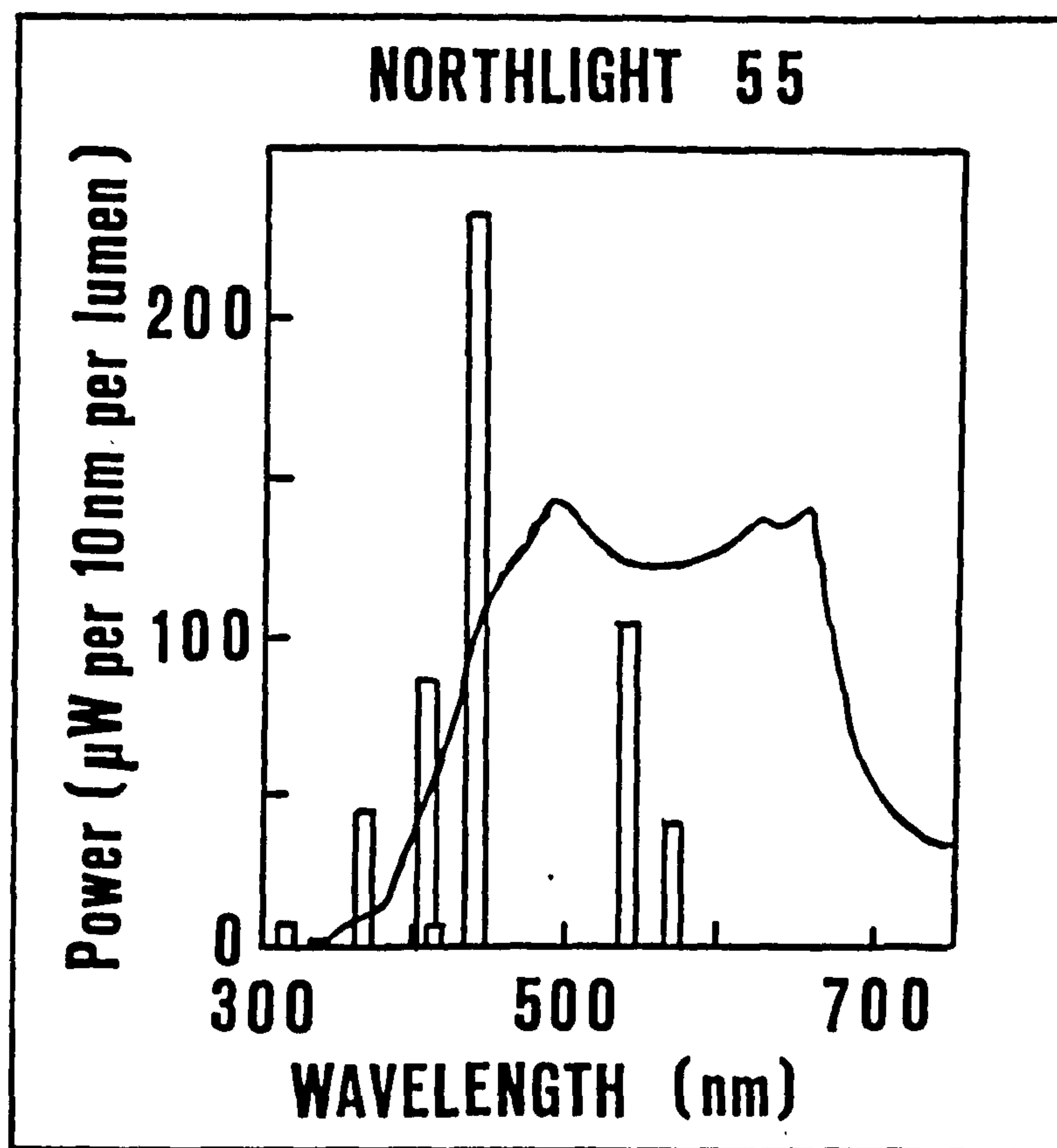


Figure 4.1 (continued) Spectral emissions of various fluorescent tubes (redrawn) [8,9].

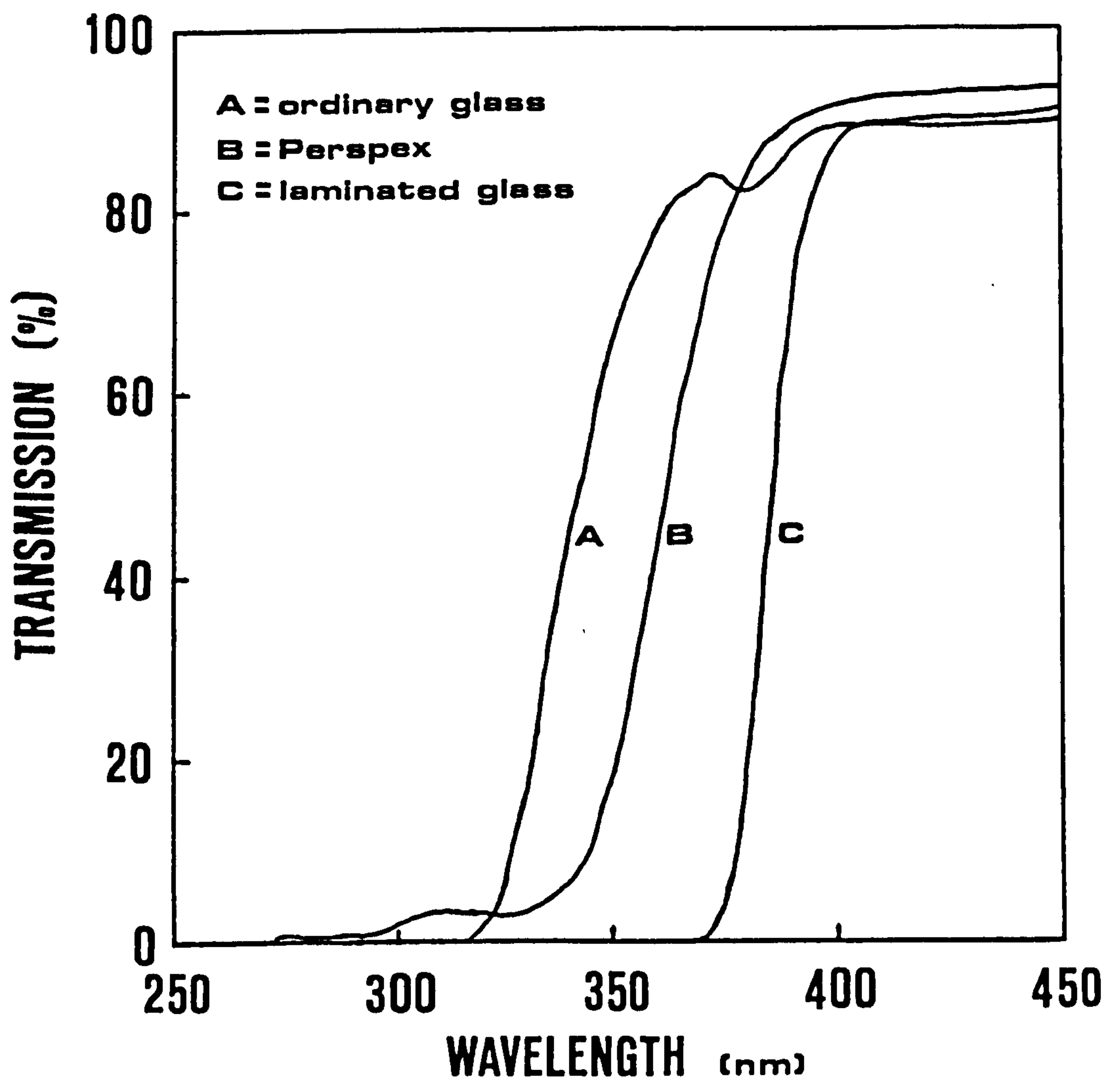


Figure 4.2 Transmission spectra of various materials.

4. 'Irradiation Method 4'. Samples were irradiated *in situ* on a Carl Zeiss LAB16 microscope using a mercury lamp (HBO 50W) with a UV or visible filter set for excitation (see Sections 4.4.2 and 4.4.2.3).
5. 'Irradiation Method 5'. Samples were irradiated *in situ* on a Carl Zeiss LAB16 microscope using an argon ion laser, model LH 1232KS (Toshiba, Japan), wavelength 488 nm, output power 10 mW (see Section 4.4.2.3).

4.3.3 Bleaching Treatments

A number of bleaching treatments were employed, which consisted of:

1. 'Bleaching Treatment 1'. Wool fabric was oxidatively bleached using the recipe:

Tetra sodium pyrophosphate decahydrate	2g.ℓ ⁻¹
Hydrogen peroxide (100 vol, 30%)	30ml.ℓ ⁻¹
Lissapol N	1g.ℓ ⁻¹

Bleaching was carried out at a liquor ratio of 25:1 and the pH adjusted to 8.5 with the addition of ammonium hydroxide. The temperature was raised to 40°C and held for 4 hours, followed by rinsing in cold water and drying at room temperature in the dark.

2. 'Bleaching Treatment 2'. Wool tops were oxidatively bleached, using the recipe:

Tetra sodium pyrophosphate decahydrate	1.5g.ℓ ⁻¹
Hydrogen peroxide (35%)	10ml.ℓ ⁻¹
Nekanil LN (BASF)	0.8g.ℓ ⁻¹

Bleaching was carried out at a liquor ratio of 20:1 and pH 9.1. The temperature was raised to 50°C in 15 minutes and held for 5 hours, followed by rinsing twice in warm water and several times in cold water before drying at room temperature.

3. 'Bleaching Treatment 3'. Wool tops were reductively bleached, using the recipe:

Blankit IN (sodium dithionite, BASF)	2.5g.ℓ ⁻¹
Nekanil LN	1g.ℓ ⁻¹

Bleaching was carried out at a liquor ratio of 20:1. The temperature was raised to 60°C in 15 minutes and held for 1 hour. The pH was adjusted to 3.7 with the addition of formic acid and held at 60°C for 20 minutes. The wool was rinsed once in warm water and several times in cold water (to the last rinse 1.2ml.ℓ⁻¹ hydrogen peroxide (35%) was added to remove sulphureous odours) before drying at 20-50°C.

4. 'Bleaching Treatment 4'. Wool tops were fully bleached. Tops which had been oxidatively bleached (Bleaching Treatment 2) but not dried were reductively bleached, using the recipe:

Blankit IN	3g.ℓ ⁻¹
Uniperol O (BASF)	0.5g.ℓ ⁻¹

Bleaching was carried out at a liquor ratio of 20:1. The temperature was raised to 60°C in 15 minutes and held for 1 hour. After 45 minutes at 60°C the pH was adjusted to 3.5 by the addition of formic acid. The wool was rinsed once in warm water and several times in cold water (to the last rinse 0.5ml.ℓ⁻¹ hydrogen peroxide (35%) was added) before drying at room temperature.

4.3.4 Separation of Morphological Components

Wool tops were separated into the morphological components - cuticle cells, cell membrane complex and cortex.

4.3.4.1 Cuticle Cells

A number of methods used for removing cuticle from keratin fibres are likely to be degradative at least to some degree [11], including treatment with formic acid with or without ultrasound. Previously formic acid treatments were a popular choice and as a preliminary to this investigation the effect of formic acid on the natural fluorescence of wool was investigated.

BA tops (6g) were shaken with 90% formic acid (150 ml) for one hour before washing with distilled water (2 x 150 ml) and air drying at room temperature. Cross sections (see Section 4.4.2.1) were examined using fluorescence microscopy (UV and violet excitation, Vickers M17 microscope, see Section 4.4.2.1). This revealed that the treatment had caused the wool fibres to have a lower mean fluorescence but with the fluorescence at the edges being more pronounced i.e. formic acid affects the fluorescence of wool. Hence, the unsatisfactory nature of formic acid for obtaining cortical cells was confirmed.

Consequently cuticle cells were liberated by mechanical treatment of fibres in a sodium dodecyl sulphate (SDS) solution after the mild method of Ley [11,12] as modified by Röper *et al.* [13]. With this method sufficient amounts of highly purified cuticle cell fragments for analytical purposes can be isolated in a short time.

Three grams of wool (cut to a length of about 3mm) were mechanically treated in a mixer (consisting of a 1.5 litre vessel with a rotating knife on the bottom) with 150 ml of a 1% w/v SDS (SERVA, research grade) solution. The total time of treatment was 10 minutes at 1 minute intervals with intermittent cooling on ice. The vessel was chilled in a refrigerator overnight (or longer), effecting the collapse of the foam. The resulting suspension was filtered through a 100 μ m steel screen and centrifuged (Beckman Model J-21C Centrifuge, 12,000 rpm, 30 min, 20°C). Residual SDS in the precipitate was removed by repeated washing and centrifuging (12,000 rpm, 30 min, 20°C), with water/ethanol, increasing the content of ethanol in each succeeding washing step. The cell fragments were suspended in pure ethanol and purified by sieving through steel screens with decreasing porosity (40,

28, 20 μm). The resulting suspension was centrifuged (Heraeus Christ Centrifuge, 3,000 rpm, 30 min, ambient temperature). The supernatant was removed by decantation and the cuticle cells dried over phosphorus pentoxide under vacuum. To check the process the first sample prepared was checked for purity using visible light microscopy and was found to be reasonably pure.

4.3.4.2 Cell Membrane Complex

Cell membrane complex (cmc) was obtained by enzymatic degradation of wool according to the method of Schwan [14].

Wool (300 mg) was placed in a glass tube with a Sovinell stopper. 25 mg of Papain (SERVA: from *Papaya carica*, 1:350 NF practical grade), 100 mg dithioerythritol (SERVA, research grade), approximately 5 mg of thymol (to stop the formation of bacteria) and 20 ml of 0.1M phosphate buffer, pH 6.7 (prepared from sodium dihydrogen phosphate monohydrate and disodium hydrogen phosphate dihydrate, both Merck analytical grade) were added. The reaction mixture was kept at 50°C for 70-85 hours with constant stirring with a magnetic stirrer and occasional shaking. The substrate must be completely covered by the solvent. Schwan found that a time of 72 hours was sufficient for complete degradation while longer times did not increase the amount of degradation.

The reaction mixture was cooled and then centrifuged (Beckman Model J-21C Centrifuge, 20,000 rpm, 30 min, 20°C). Three times the residue (i.e. the cmc) was washed with water. Each time the solution was centrifuged. The resulting residue was resuspended and freeze dried.

4.3.4.3 Cortex

Difficulty was experienced in obtaining a suitable process for obtaining pure cortex. Initially, the method of Wortmann *et al.* [15] as modified by Sakabe *et al.* [16,17] was tried. This involved removal of the cuticle cells from wool fibres by shaking with glass fibre snippets in aqueous propanol.

Glass fibre snippets (40-100 μm) were obtained by grinding fibre glass followed by filtering and then drying at 100°C for 1.5 hours. 0.1g of wool fibres (cut to a length of about 5 mm) was added to 2g of the glass fibre snippets (giving a wool:glass fibre mass ratio of 1:20). 50 ml of n-propanol/water (1/1, v/v) was added and the mixture mechanically shaken for 40 hours. A mixture of wool fibre components and glass fibre snippets was recovered by centrifugation (Heraeus Christ Centrifuge, 3,000 rpm, 20 min, ambient temperature). This was washed twice with deionized water and twice with ethanol, with centrifugation (3,000 rpm, 20 min, ambient temperature) after each washing. The mixture was dispersed in a carbon tetrachloride/ethanol (1:1) mixture and centrifuged (3,000 rpm, 20 min, ambient temperature). Centrifugation failed to separate the wool fibre components from the glass fibre snippets effectively. After filtration with a 100 μm steel screen, checking with visible light microscopy revealed that very few of the fibres had had their cuticles removed.

As this method did not appear very successful three shaking methods were then tried concurrently, involving glass fibre snippets (again), (whole) glass fibres [17] and carborundum [18,19].

For the first method, the glass fibre snippets were obtained by shaking glass fibres in water for 2 hours followed by drying at 100°C for one hour. The snippets were not filtered but large pieces of glass fibre remaining were removed by hand. 0.1g of wool fibres (cut to a length of about 3 mm) was added to 2g of the glass fibre snippets. 50 ml of n-propanol/water (1/1, v/v) was added and the mixture mechanically shaken for 65 hours. Checking with visible light microscopy revealed that a few fibres had some cuticle cells removed. The mixture was shaken for a further 45 hours and then examined again. It was observed that some fibres were completely decuticled (though often with split ends), some fibres had no cuticle removed, and some fibres had most of their cuticles removed. Hence, this process was not ideal as the resulting wool fibres were very inhomogeneous.

For the second method, 2.2g of glass fibres were added to 0.1g of wool fibres (cut to a length of about 3 mm). 50 ml of n-propanol/water (1/1, v/v) was added and the mixture mechanically shaken for 65 hours. Checking with visible light microscopy revealed that quite a few fibres were mostly decuticled. The mixture was shaken for a further 45 hours and then examined again. It was found that most fibres were completely decuticled and also quite a few had split ends.

The third method utilised carborundum (silicon carbide) as the abrasive. 3.2g of carborundum was added to 0.1g of wool fibres (cut to a length of about 3 mm). 50 ml of n-propanol/water (1/1, v/v) was added and the mixture mechanically shaken for 25-65 hours (the exact time is not known as the container fell off the shaker while unattended). Checking with visible light microscopy revealed that the occasional fibre was partly decuticled. The mixture was shaken for a further 45 hours and then examined again. It was observed that nearly all the fibres were completely devoid of cuticle and none had split ends. The wool cortexes were then separated from the carborundum. The mixture was allowed to settle (overnight) before the liquid was decanted. Some of the wool fibres had felted. These were discarded. The remaining wool cortexes were picked out in groups by tweezers and then washed on a 100 μm steel screen with copious amounts of distilled water. The sieve retained the carborundum as well as the cortexes so manual separation with tweezers ensued. The washed cortexes were then dried over phosphorus pentoxide under vacuum.

Clearly the carborundum method was the best of the three methods tried and was subsequently used to obtain cortexes of other wool fibres. All of the foregoing attempts had used DWI tops; cortexes were then also obtained using DWI tops which had been bleached (oxidatively, reductively and fully, which had been prepared using Bleaching Treatments 2, 3 and 4 respectively with the assistance of Dr. K. Schäfer (DWI) (see Section 4.3.3)). These preparations were carried out at ambient temperature which varied widely and meant that cooling was sometimes used. Also (by accident) iso-propanol was used instead of n-propanol. The mixtures were shaken and checked periodically by visible light microscopy until satisfactory decuticled cortexes were obtained (35-60 hours).

4.3.5 Amino Acid Analysis

To the sample (of wool fibre or morphological component) (usually 1-2 mg, sometimes 3-5 mg) was added 6N hydrochloric acid (analytical grade). The mixture was frozen using ethanol/solid carbon dioxide before being warmed to room temperature under vacuum (5-10 min) to remove dissolved oxygen. The sample tube was sealed under vacuum and then kept at 110°C for 24 hours.

The sample solution was transferred to a flask and evaporated to dryness under reduced pressure. Subsequent washing with water and evaporation to dryness was carried out three times. The remaining part of the analysis was carried out by Dr J. Föhles of the DWI.

4.4 FLUORESCENCE EXAMINATIONS/MEASUREMENTS

The fluorescence of samples was observed using a UV tlc lamp, a spectrophotometer and by microscopy.

4.4.1 Fluorescence Spectrophotometer (MPF-4)

A Perkin-Elmer Fluorescence Spectrophotometer (MPF-4) (fluorimeter) was used to obtain fluorescence measurements, which are uncorrected. The spectrophotometer can measure either liquids or solids. The attachment for solids was designed and made at City University and is similar to that described by McKellar and Allen [20]. The samples are placed at 50° to the incident beam to avoid scattered light.

4.4.2 Fluorescence Microscopy

A number of fluorescence microscopes were used. All used high pressure mercury lamps (50 or 100 W) for illuminating the sample. A mercury lamp emits a characteristic line spectrum (Figure 4.3), including lines at 366, 405, 436, 546 and 578 nm [21]. High pressure lamps also have a strong background continuum. The filters used are described with the appropriate microscope.

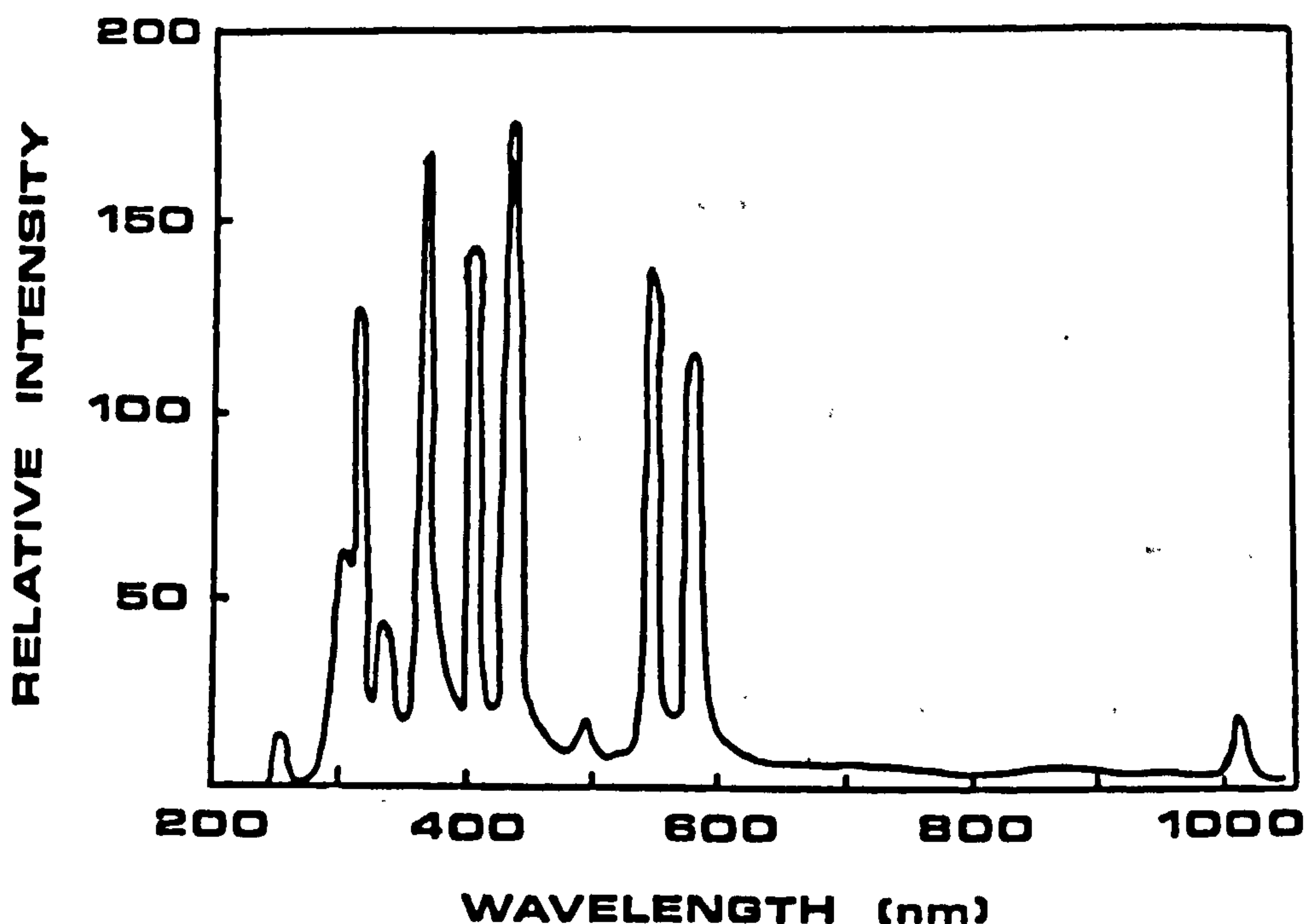


Figure 4.3 Emission spectrum of a high pressure mercury lamp (HBO 100W) (redrawn) [21].

4.4.2.1 M17 Fluorescence Microscope

A Vickers M17 incident light fluorescence microscope was used at IWS with the assistance of Dr P.H. Greaves. A high pressure mercury lamp, HBO 50W, was used with two filter sets - 'UV' and 'violet'.

Figure 4.4 is a schematic diagram of the microscope set-up for excitation with violet light. With violet excitation mode, excitation is with broad band light of wavelength 350-400 nm. The dichroic reflector transmits light of wavelength >455 nm and the barrier filter transmits light of wavelength >475 nm. To the eye, the fluorescent samples examined appear green. (The barrier filter prevents any stray UV light reaching the eye). With UV excitation mode, excitation is with narrow band radiation of peak wavelength 365 nm. The dichroic reflector transmits light of wavelength >430 nm and the barrier filter transmits light of wavelength >420 nm. To the eye, the fluorescent samples examined appear blue.

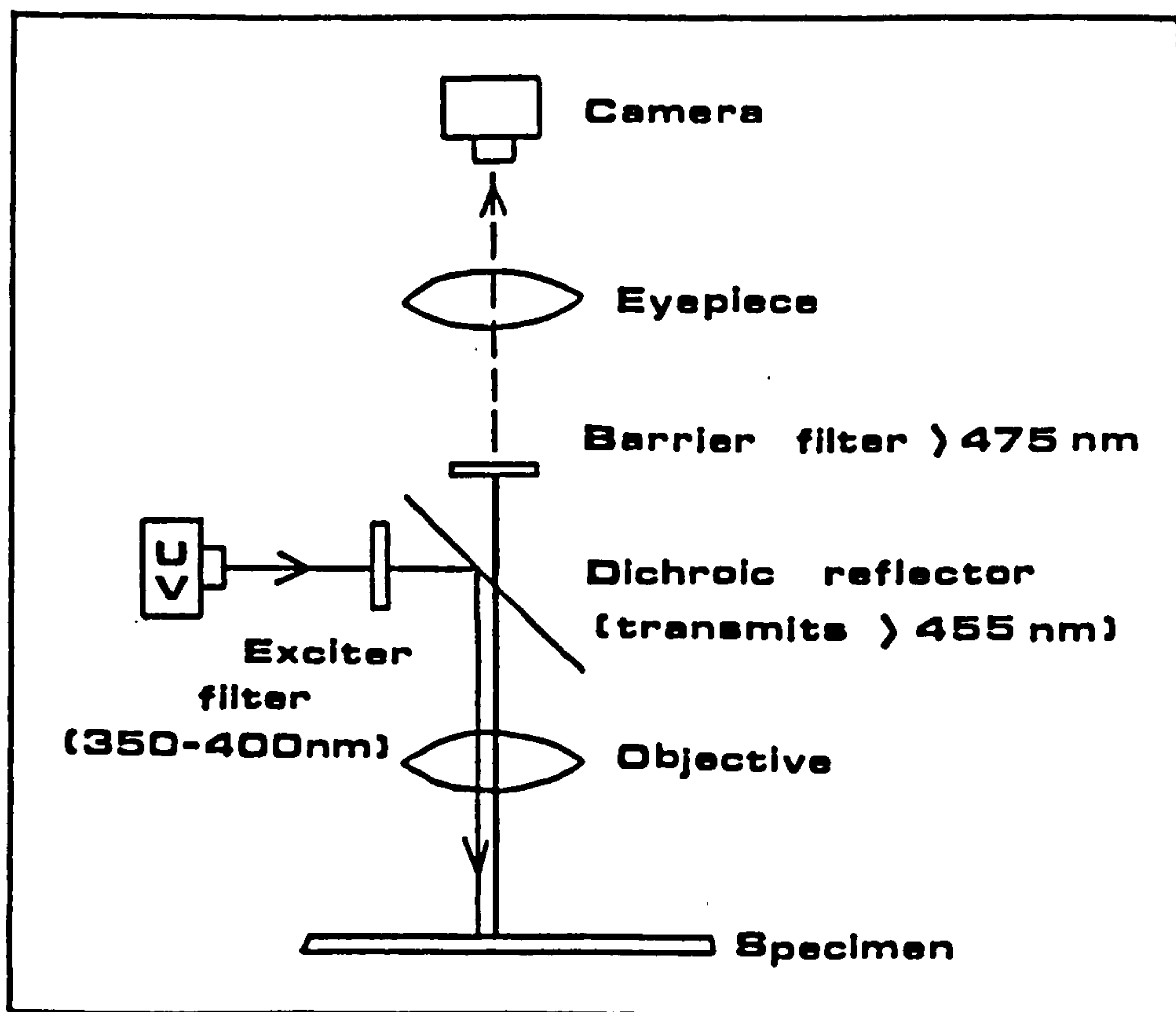


Figure 4.4 Schematic diagram of a Vickers M17 fluorescence microscope (violet excitation mode) (altered) [22].

Cross sections of fibres were prepared using a Hardy microtome [23]. This is a small microtome ideal for cutting fibre cross sections. Fibres from fleece, yarn, fabrics etc. were placed in the Hardy microtome. 6% cellulose acetate in acetone was used to embed the excess fibres which were subsequently removed with a scalpel. The same adhesive was used to embed fibre cross sections as they protruded from the microtome. The cross sections were cut with a scalpel and then 'cleared' by being placed on xylene on a glass slide. They were examined with a Vickers M72 transmitted light microscope. Satisfactory cross sections were placed on xylene on a new glass slide, approximately 0.5 ml of Gurr's Neutral Mounting medium (BDH) was added and a coverslip placed on top. After allowing the mountant to dry the cross sections were ready for fluorescence microscopy. When cross sections were prepared using fibres from uncleaned fleece xylene was not used.

Photomicrographs on the Vickers M17 were taken using a 25/0.5 objective and an Olympus OM4 camera. Magnification was checked by photographing a stage micrometer under the same conditions as the specimens.

4.4.2.2 Scanning Photometer Microscope

A Carl Zeiss Scanning Photometer Microscope 03 with MPC 64 control unit (Carl Zeiss, Oberkochen, Germany) was used at DWI with the assistance of Dr K. Schäfer. A high pressure mercury lamp, HBO 100W, was used together with a UV filter set. This comprised excitation band pass filter G365, selective mirror FT395 and emission long pass filter LP420 (for more details see Section 4.4.2.3 and Figure 4.6).

The Scanning Photometer Microscope uses incident-light excitation. Hence, the microscope configuration is the same as in Figure 4.4 but with the filters changed accordingly and the camera replaced by the photometer.

4.4.2.3 LAB16 Fluorescence Microscope

A LAB16 incident light fluorescence microscope (Carl Zeiss, Oberkochen, Germany) was used, initially with the assistance of Dr M.E.C. Hilchenbach. Figure 4.5 is a schematic diagram of the microscope and most of its associated equipment. A range of facilities are available and are described here.

Three excitation sources were used - a helium cadmium laser, an argon ion laser and a mercury lamp. The main excitation source was the high pressure mercury lamp, HBO 50W, which was used with two filter sets - 'UV' and 'visible' (the latter officially designated 'blue' by Zeiss). Additionally a heat absorbing filter KG1 and a red-attenuating filter BG38 were present in the leading light path.

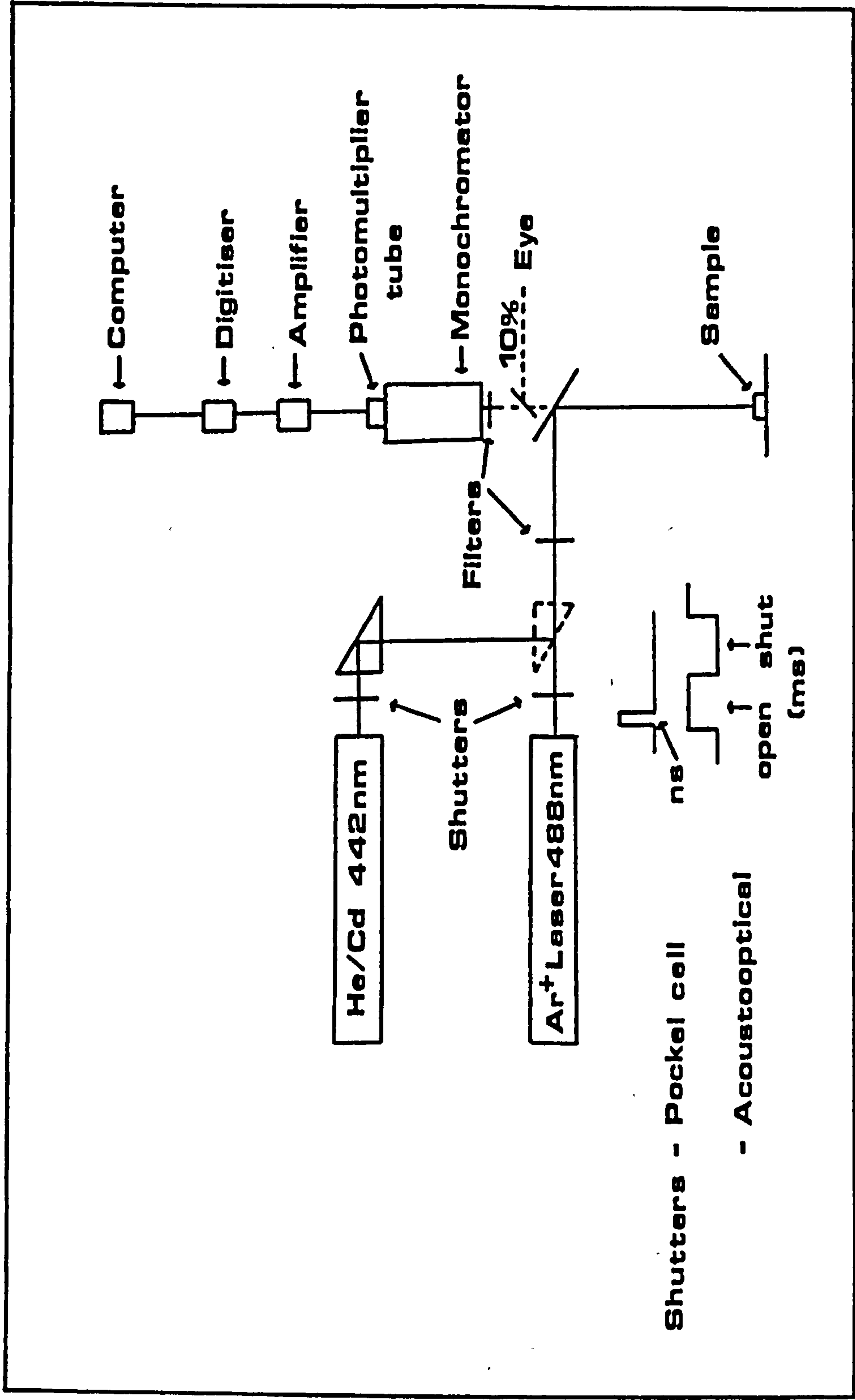


Figure 4.5 Schematic diagram of LAB16 microscope and associated equipment (altered) [24].

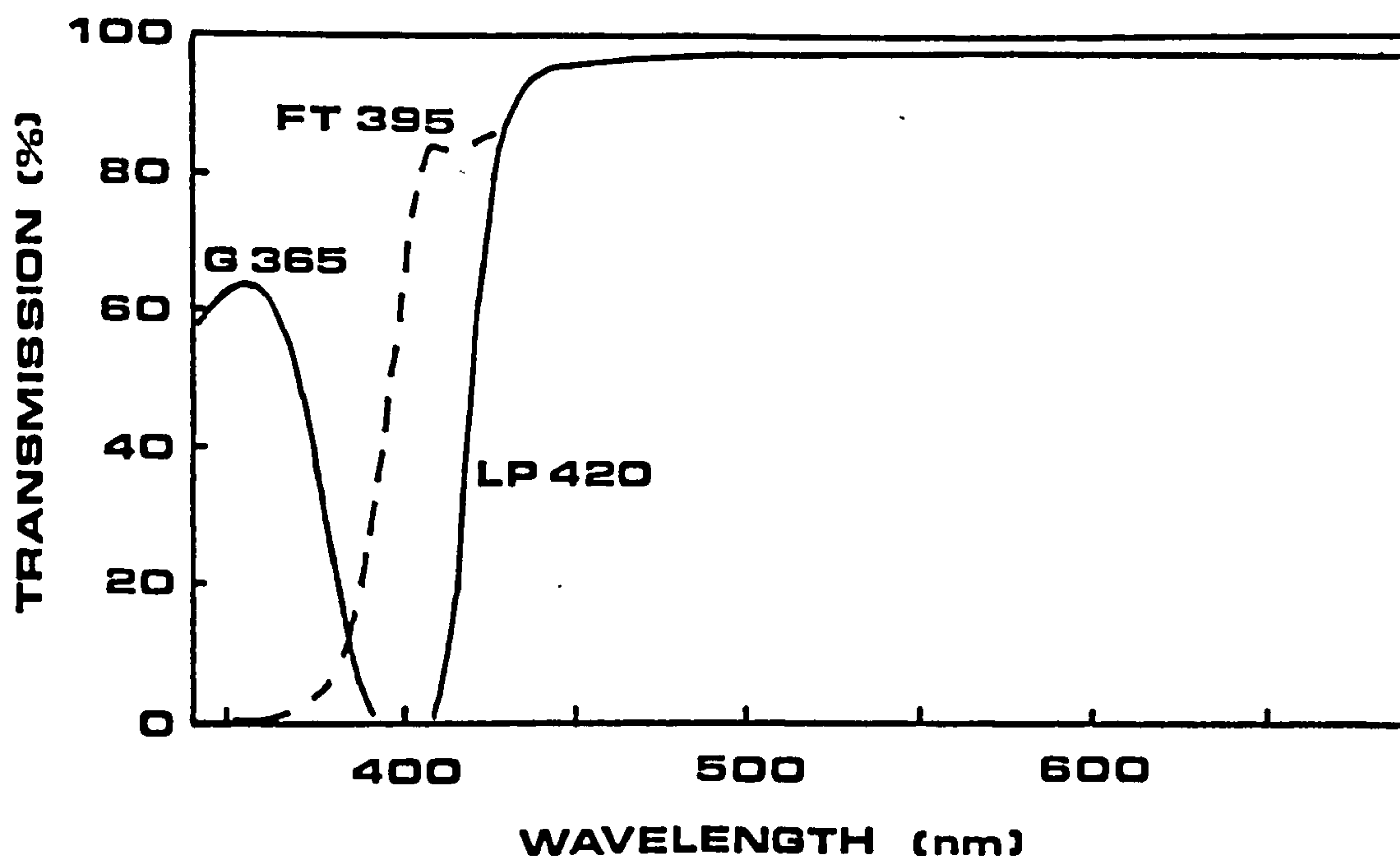


Figure 4.6 Transmittance spectra of UV filter set (LAB16) (redrawn) [25].

With UV excitation mode, excitation band pass filter G365, selective (i.e. dichroic) mirror FT395 and emission long pass filter LP420 were used. To the eye, the fluorescent samples examined appear blue. Figure 4.6 shows the transmittance spectra of the UV filter set (Zeiss number 02).

With visible excitation mode, excitation band pass filter BP 450-490, selective mirror FT510 and emission long pass filter LP520 were used. To the eye, the fluorescent samples examined appear green. Figure 4.7 shows the transmittance spectra of the visible filter set (Zeiss number 09).

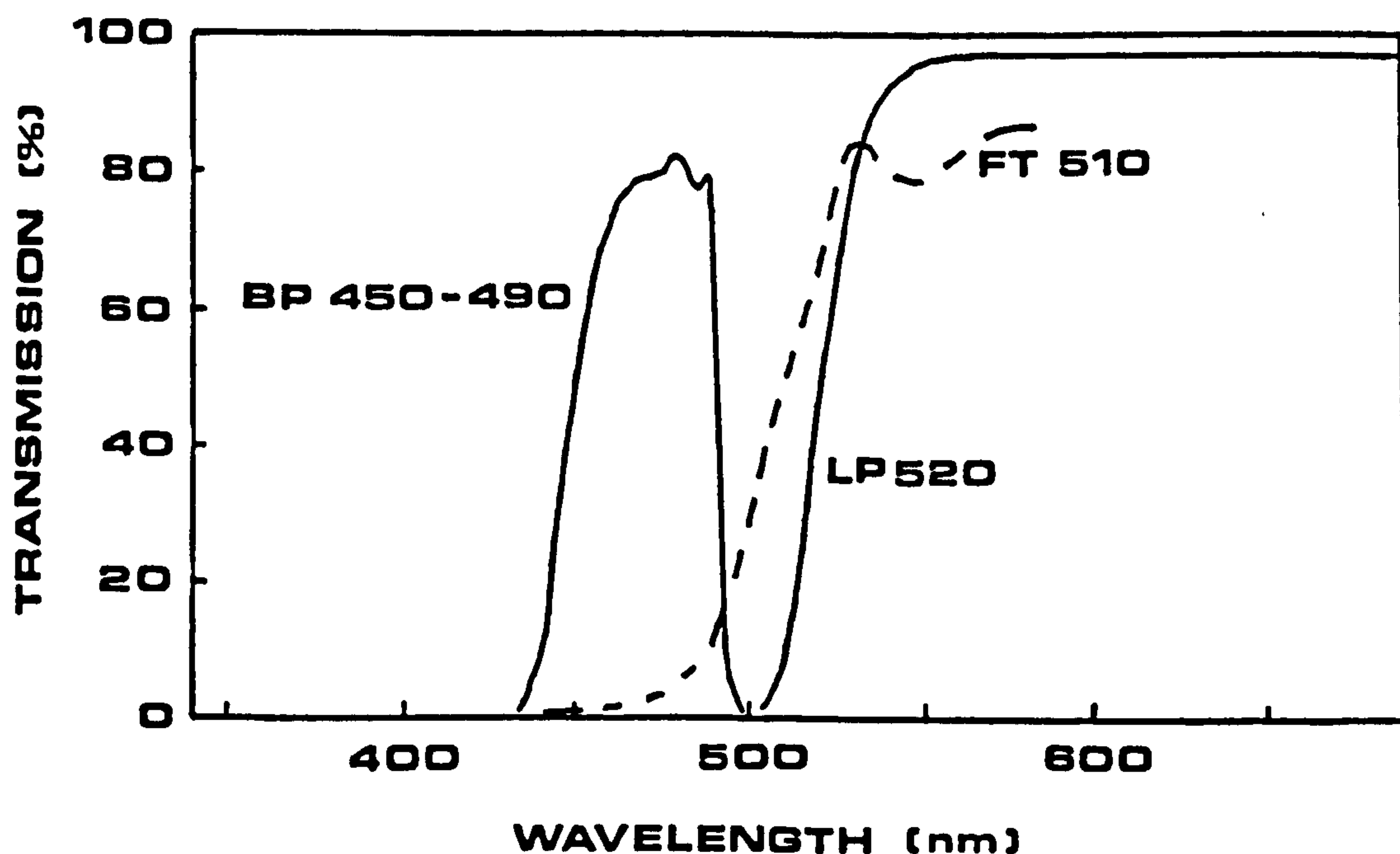


Figure 4.7 Transmittance spectra of visible filter set (LAB16) (redrawn) [25].

As well as the mercury lamp, two lasers were used as excitation sources. They were a helium cadmium laser, model 4240 NB (Liconix, Sunnyvale, USA), wavelength 442 nm, output power 50 mW, and an argon ion laser, model LH 1232 KS (Toshiba, Japan), wavelength 488 nm, output power 10 mW. For both lasers only the visible filter set was used, with the excitation band pass filter BP 450-490 removed. The (continuous wave) lasers were gated by an acoustooptical shutter device, rise time 50 ns, (A.A. France), controlled by a microcomputer C64 (Commodore, Braunschweig, Germany). The diameter of the laser beam impinging on the sample was 4 μ m.

Once the exciting source had caused the sample to fluoresce the fluorescence could be monitored by one of three ways: visually, photographically and numerically. On the LAB16 is a trinocular head (which allows an attachment to be added) and an associated mirror. The mirror allows either 100% of the light to go to the eye piece or 90% to the attachment and 10% to the eye piece. Two attachments were

used - a camera or a 'photometer' (as shown in Figure 4.5). The camera, an Olympus OM2, allowed photomicrographs to be obtained. Magnification was checked by photographing a stage micrometer under the same conditions as the specimens. The photometer allowed fluorescence emission spectra to be recorded and fluorescence intensities to be measured, on a single spot (confocal focusing). The fluorescence emission spectra were recorded with a monochromator, grating 1200 grooves/mm (Bausch & Lomb, USA). The fluorescence intensities were determined with a photomultiplier tube R446 (Hamamatsu, Japan), connected to a Tektronix digitiser TD20T (Beaverton, USA) and/or via an 8 bit A/D interface to a microcomputer. Initially for comparing fluorescence intensities of samples, fluorescence intensities were measured at single wavelengths using the monochromator. However, the natural fluorescence of wool produces quite a weak signal so the technique was changed to measuring all the fluorescence produced by a sample.

The objectives used were a 5/0.15 H8237 (Vickers), a 10/0.25 N4788 Microplan (Vickers), a 16/0.40 Neofluar (Zeiss), a 32/0.4 F-Achromat LD (Zeiss) and a 40/0.75 Neofluar (Zeiss), where the first number e.g. 40 is the magnification of the objective, and the second number e.g. 0.75 is the numerical aperture.

The choice of objective depended on the nature of the sample being investigated. For example, it was found that fabrics (which have many fibres one on top of the other) had a low depth of field (of view), requiring a 5x objective. Consequently further fluorescence measurements of whole fabrics were restricted to the fluorescence spectrophotometer (see Section 4.4.1). However, a 16x objective was the normal choice for inspecting whole fibres, while a 40x objective could be used on good cross sections. (The depth of field of an objective is inversely proportional to the numerical aperture.)

All the samples investigated (except single wool fibres) were first embedded on a glass slide. Embedding involved placing the sample on the glass slide, adding one or more drops of embedding medium and then pressing a coverslip (0.17 mm thick) on top. Initially, a few different embedding media were tried. Glycerol (AnalaR, BDH) was found to be the best and was then used exclusively.

Cross sections of fibres were prepared using a Hardy microtome, as described in Section 4.4.2.1, except that once they had been prepared they were not examined with visible light microscopy but embedded immediately in glycerol.

4.4.2.4 Inverted Fluorescence Microscope (IM)

A Carl Zeiss (Oberkochen, Germany) Inverted Microscope IM, an inverted incident light fluorescence microscope, was also used. This replaced the LAB16 microscope (see Section 4.4.2.3) and acquired all the optics and accessories that the LAB16 had. For more information on these see Section 4.4.2.3.

4.5 OTHER MEASUREMENTS

4.5.1 CIE Tristimulus/Yellowness Index Measurements

The CIE tristimulus values, X, Y and Z, were obtained using an ICS-TEXCON Macbeth Micromatch Reflectance Spectrophotometer (2000 or 2020 plus) (D65/10° illuminant/observer). The CIE tristimulus values were used to calculate the Yellowness Index (YI) measurements, according to the following formula:

$$YI = \frac{100(1.316X - 1.164Z)}{Y}$$

The lower the YI value, the whiter is the wool. The determination of colour is considered further in Section 2.4.3.1.

4.5.2 Diffuse Reflectance Spectroscopy

Diffuse reflectance spectra of fabric samples were obtained using a Perkin-Elmer Lambda 5 UV/vis spectrophotometer, equipped with an integrating sphere. Barium sulphate was used as the reference.

4.5.3 UV/visible Absorption Spectroscopy

UV/visible absorption spectra were obtained using either a Perkin-Elmer Lambda 5 UV/vis spectrophotometer or a Philips PU8720 UV/vis scanning spectrophotometer. These spectrophotometers were also used to make solutions up to a known optical density. Measurements of solutions were made using UV grade quartz cells of path length 1cm.

4.5.4 Infra-Red Spectroscopy

Normal infra-red spectra were obtained using a Perkin-Elmer 983G instrument. The spectra of wool greases were recorded after having spread the grease between a pair of sodium chloride discs. Fourier transform infra-red (FTIR) spectra were recorded using a Bio-Rad FTS 60.

4.5.5 Statistics

(In this subsection 's' is used as the normal statistical abbreviation for standard deviation. In the rest of this thesis 's' is used as the SI symbol for second.)

On a number of occasions the fluorescence intensities of different samples have been compared quantitatively [26]. The fluorescence intensity of a sample has been recorded as the average value (given by the mean) and the degree of spread (given by the standard deviation and/or standard error of the mean). The mean, \bar{x} , is the sum of all the measurements divided by the number of measurements, n:

$$\bar{x} = \frac{\sum_1^n x_i}{n}$$

The standard deviation, s, (which is an absolute measure of the spread of the measurements) is given by the formula:

$$s = \sqrt{\frac{\sum_i (x_i - \bar{x})^2}{(n-1)}}$$

The standard error of the mean, sem, (which is a measure of the accuracy of the mean) is given by the formula:

$$sem = \frac{s}{\sqrt{n}}$$

Random errors make it very unlikely that the fluorescence intensity of two samples would have the same measured values even if they had the same real values. To decide whether the differences between two samples can be accounted for by random errors a significance test is used which finds out if the differences are significant. The test used for comparing two sample means was the t-test. However, before this can be done the standard deviations of the two samples need to be compared to see if they differ significantly. (Both the t-test and the F-test assume that the distribution of the values from each of the individual samples take a normal (or Gaussian) distribution, which is a standard statistical assumption.)

The F-test considers the ratio of the two sample variances, i.e. the ratio of the squares of the standard deviations. The quantity calculated (F) is given by:

$$F = \frac{s_1^2}{s_2^2}$$

s_1^2 and s_2^2 being allocated in the equation so that F is always ≥ 1 . The number of degrees of freedom is n_1-1 , n_2-1 and a two-tailed test is carried out.

The t-test is then carried out on the two sample means, \bar{x}_1 and \bar{x}_2 . If the two samples have standard deviations which are not significantly different, a pooled estimate of standard deviation can be calculated from the two individual standard deviations s_1 and s_2 by using the equation:

$$s^2 = \frac{((n_1-1)s_1^2 + (n_2-1)s_2^2)}{(n_1 + n_2 - 2)}$$

t is then given by:

$$t = \frac{(\overline{x_1} - \overline{x_2})}{s \sqrt{\frac{1}{n_1} + \frac{1}{n_2}}}$$

where t has $(n_1 + n_2 - 2)$ degrees of freedom. However if the assumption that the population standard deviations are equal is not valid, then t is given by:

$$t = \frac{(\overline{x_1} - \overline{x_2})}{\sqrt{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}}}$$

and the number of degrees of freedom is calculated from:

$$\text{degrees of freedom} = \left\{ \frac{\left(\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2} \right)^2}{\frac{\left(\frac{s_1^2}{n_1} \right)^2}{n_1 + 1} + \frac{\left(\frac{s_2^2}{n_2} \right)^2}{n_2 + 1}} \right\} - 2$$

the result being rounded to the nearest whole number.

The more measurements that are made per sample the more tedious the procedure becomes and the smaller the standard deviation becomes, enabling differences between samples to be observed more easily. Differences in homogeneity were found to occur between different types of sample examined. Fibres from yarns, as well as tops and threads from fabrics, were very inhomogeneous. To overcome this 40 or 50 measurements were made per sample. On fibres from fleece (of the Merino or Merino type where weathering is restricted to the fibre tips) fluorescence intensities were measured at the middle of the fibre so greatly reducing the influence of the weathering already present. For these samples initially 15 measurements were made per sample but due to some inconclusive results (subsequently shown to be negative) this was increased for later examinations.

To reduce random error, the order in which the samples were measured for a particular experiment was randomised.

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CHAPTER 5

NATURAL FLUORESCENCE OF WOOL -

CHARACTERISATION

5.1 INTRODUCTION

It is well known that white wool photoyellows and tenders on exposure to sunlight (see Section 2.4, especially Section 2.4.3.2) and consequently the material cannot be used in a number of products. If the photoyellowing and phototendering could be retarded, or even better eliminated, wool would be in a better position to compete with many man-made fibres.

Studies on the photoyellowing of wool have shown that the photo-oxidation of the amino acid tryptophan is of great importance (see Section 2.4.7). The tips of wool fleeces are photoyellowed much more than the roots since the tips suffer far more exposure to sunlight (see Sections 2.4.1, 2.4.3.3. and 2.4.8). It is to be expected therefore, that there exists a correlation between the occurrence of (fluorescent) tryptophan degradation products and the position along the length of a wool fibre. Prior to the course of this investigation there appears to be only a brief report published concerning this. Hirst in 1927 [1] observed that Australian Merino greasy wool exhibited a fluorescence which was yellow in colour with bluish-white tips (see Section 2.5.1). The lack of further reports is surprising, as simple qualitative experiments (using a UV tlc lamp) readily reveal that the natural visible fluorescence of Merino fibres vary from highly fluorescent tips to barely fluorescent roots [2].

This chapter describes a series of measurements which have been carried out in order to quantify the variation in natural fluorescence along a (Merino) fibre and to identify aspects of the wool fibre morphology and composition which contribute to the fluorescence observed.

5.2 EXPERIMENTAL

A detailed explanation of the main materials and techniques used can be found in Chapter 4.

5.2.1 Variation in Natural Fluorescence Along Fibres

Fibres from Merino fleece 1 (average diameter $21.5\mu\text{m}$) were cleaned using Cleaning Method 1 (shaking with tetrachloroethene). Snippets were obtained from the staples (average length 65 mm) of tip ends (first 5 mm of staple), middle portions (30-40 mm of staple) and root ends (last 10 mm of staple). Cross sections were prepared using a Hardy microtome and photomicrographs obtained using both UV and violet excitation modes with a 25/0.5 objective of a M17 fluorescence microscope (Section 4.4.2.1). By trial and error it was found that the best images showing the natural fluorescence were obtained using camera exposure times of 60 seconds (Kodachrome 64 ASA Professional (Daylight) colour transparency film). Cross sections and photomicrographs of BA tops were prepared similarly.

Fibres from Merino fleece 1 were cleaned using Cleaning Method 1. A single wool fibre (approximately 6 cm long) was embedded on a glass slide using immersion oil (Olympus) and examined using a LAB16 fluorescence microscope equipped with a 40/0.75 Neofluar objective (Section 4.4.2.3). (The coverslip was not big enough to cover the whole length of the fibre.) An argon ion laser (wavelength 488 nm) was used for excitation and the fluorescence emission monitored at 550 nm. Measurements were made at approximately 0.2 mm intervals except for the first 1.4 mm (tip end) where they were made at approximately 0.1 mm intervals.

5.2.2. Comparison of Fluorescent Species From Tips and Roots

Fibres from Merino fleece 1 were cleaned using Cleaning Method 1. Portions of tips and roots were embedded in glycerol/carbonate buffer solution. Fluorescence emission spectra were obtained using a LAB16 fluorescence microscope equipped with a 40/0.75 Neofluar objective (Section 4.4.2.3). UV excitation mode was used.

Fibres from Merino fleece 1 were cleaned using Cleaning Method 1. Portions of tips and roots were embedded in glycerol for studying the 'fading' and recovery of the fluorescence intensity. The fading of the tips and roots is the decrease of the fluorescence intensity during laser irradiation and the recovery is the increase in fluorescence intensity after switching off the continuous irradiation, measured by repetitive laser pulses (pulse length $1.1\mu\text{s}$, pulse-dark interval 2.3 ms). A helium cadmium laser (wavelength 442 nm) was used with the fluorescence being monitored at 550 nm using a LAB16 fluorescence microscope equipped with a 40/0.75 Neofluar objective (Section 4.4.2.3).

5.2.3 Effect of Disulphides

The fluorescence quenching role of the disulphides was examined by reduction (pad-batch) and alkylation. Padding was carried out on a laboratory size Peter Pad (Benninger) with the horizontal nip forming the pad trough. Batching was carried out by rolling up the tops on a cylinder, covering with Polythene film and storing for the required time at room temperature.

BA tops were reduced using a pad liquor containing:

Sodium borohydride	20g. ℓ^{-1}
Lissapol N	10g. ℓ^{-1}
Sodium hydroxide solution	0.1M

giving a pH of 12.6. The wet pick-up level was 60% giving 1.2% on weight of fibre. The tops were stored overnight at room temperature. Half of the sample was rinsed in cold water and dried at room temperature. The other half was then further treated for 72 hours in a solution containing:

Sodium monochloroacetate 67g.ℓ⁻¹

Lissapol N 7g.ℓ⁻¹

having a pH of 8.7. The tops were then rinsed in cold water and dried at room temperature.

Fluorescence intensities of the samples were obtained using a fluorescence spectrophotometer (MPF-4) (Section 4.4.1). The samples were excited at 370 nm and the fluorescence emission measured between 330 and 730 nm. For each sample three portions were measured. Due to the 'fluffy' nature of tops very great care had to be exercised in putting portions of tops into the solids attachment of the spectrophotometer. Consequently, the use of tops for experiments (involving fluorescence spectrophotometer measurements) fell into disuse.

5.2.4 Morphological Components

DWI tops were separated into their morphological components (Section 4.3.4). DWI tops which had been bleached (oxidatively, reductively and fully using Bleaching Treatments 2, 3 and 4 respectively) were also separated into their morphological components.

Fluorescence spectra of morphological components (embedded in immersion oil) were obtained using a Zeiss Scanning Photometer Microscope with UV excitation and a Ultrafluar 10/0.20 objective (Section 4.4.2.2). The fluorescence intensities of the morphological components (embedded in a glycerol/water (3:1) solution) were obtained using a LAB16 fluorescence microscope equipped with a 40/0.75 Neofluar objective (Section 4.4.2.3). The samples were excited with an argon ion laser (488 nm, 10 mW) and the fluorescence monitored at 550 nm. The results are the average of 15 measurements. Infra-red spectra of the morphological components were recorded using a FTIR spectrophotometer equipped with a diffuse reflectance attachment. Amino acid analyses of the components were also obtained.

5.3 RESULTS AND DISCUSSION

5.3.1 Variation in Natural Fluorescence Along Fibres

As already described in the introduction, simple qualitative experiments (using a UV tlc lamp) readily reveal that the natural visible fluorescence of Merino fibres vary from highly fluorescent tips to barely fluorescent roots [2].

Cross sections of tip, middle and root portions of cleaned Merino staples were prepared and fluorescence micrographs produced using both UV and violet excitation modes. The most intense fluorescence was obtained using the violet excitation mode and therefore, only these results were reproduced in Figure 5.1 (a-c).

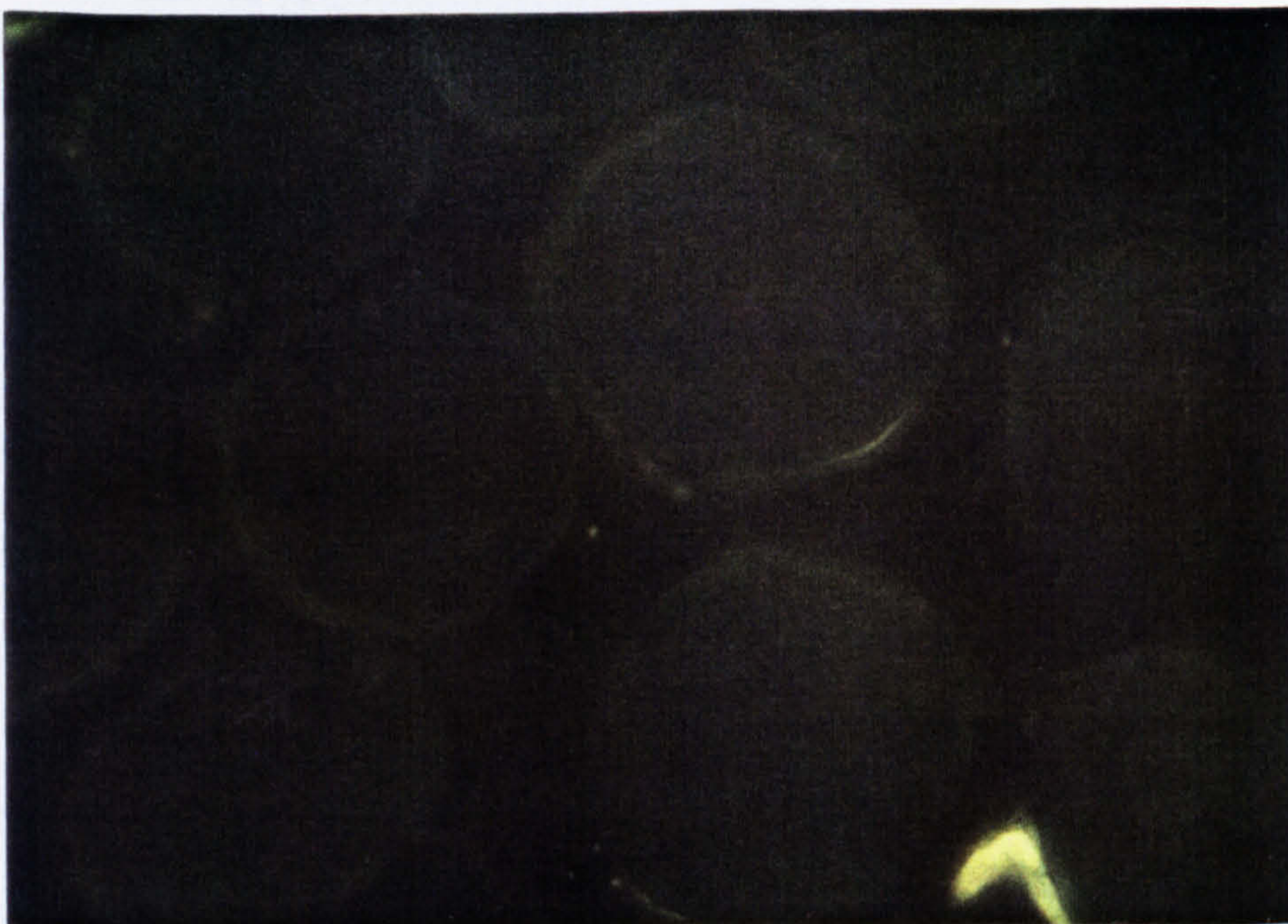
A study of Figure 5.1 shows practically zero emission from the roots, edges (or cuticle) fluorescence developing in the middle region and pronounced fluorescence in the tip region. In the tip region it is possible to make out certain interesting structures; the cuticle/cortex cell boundary is clearly visible, the orthocortex appears slightly more fluorescent than the paracortex and areas of high fluorescence show as regular dots or granules. The presence of granules of localised high fluorescence is difficult to explain, but it may be that they are either associated with cell nuclear remnants or represent complex molecules that are melanin precursors (a sort of genetic reminder that once all sheep's wool was black or brown, being coloured by discreet melanin granules).

This clear demonstration that the tips of Merino wool fibres are highly (visibly) fluorescent may be of use in objective measurements where there is a demand to quantify the tippiness of wool.

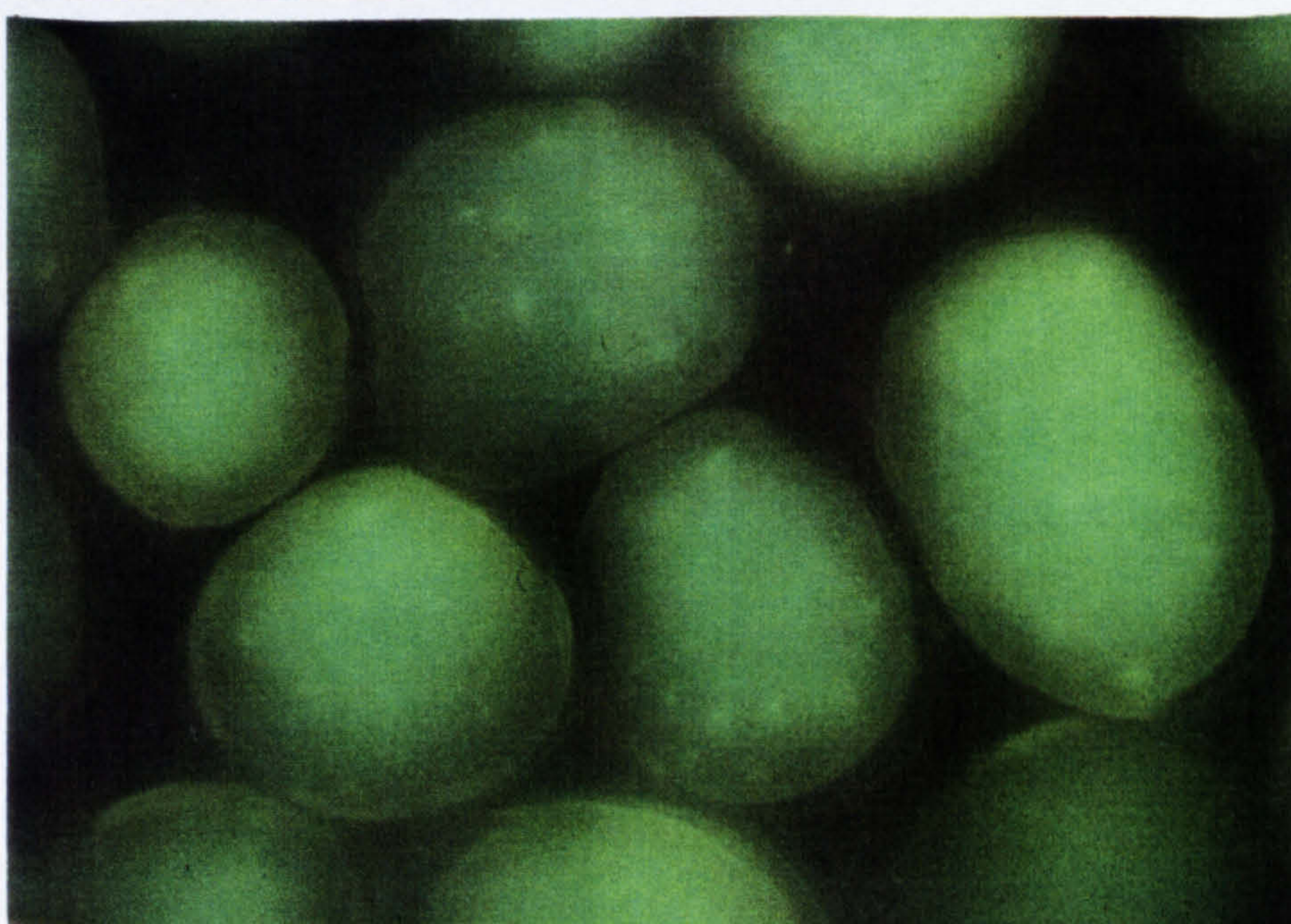
A commercially available Buenos Aires Lincoln wool top was also sectioned and a visible fluorescent micrograph obtained (Figure 5.2). From this picture the distribution of highly fluorescent fibre tips right through to virtually non-fluorescent roots can clearly be seen. Once again the spotty or granular nature of the fluorescence in the cortex is marked.



(a) root



(b) middle



(c) tip

Figure 5.1
(PTO)

Figure 5.1

- (a) Fluorescence micrograph of a cross section of the root region of a cleaned Merino wool staple (violet excitation, magnification x1060).
- (b) Fluorescence micrograph of a cross section of the middle region of a cleaned Merino wool staple (violet excitation, magnification x1060).
- (c) Fluorescence micrograph of a cross section of the tip region of a cleaned Merino wool staple (violet excitation, magnification x1060).

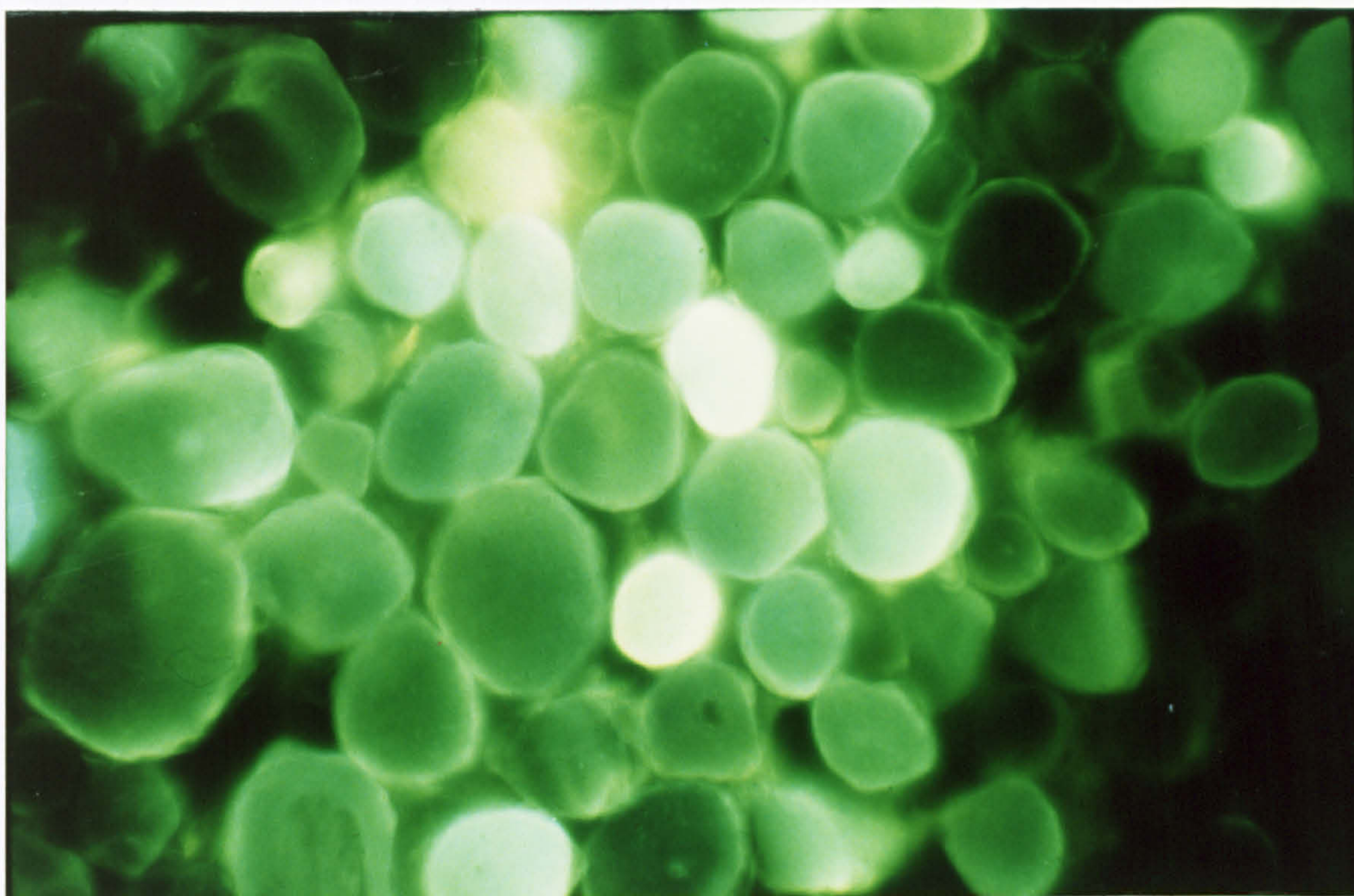


Figure 5.2 Fluorescence micrograph of a cross section of Buenos Aires Lincoln wool top (violet excitation, magnification x360).

To more accurately quantify the variation in the fluorescence from tip to root the fluorescence intensity along single wool fibres was measured. The result from one fibre is shown in Figure 5.3. The decrease in fluorescence on going along the fibre from tip towards the middle region is very rapid, with the first 5 mm being the most intense. There is then a relatively small decrease on going along the fibre from the middle region to the root. Compared with the root, the tip is about ten times more intense. (Other fibres inspected showed different ratios.) Although the degree of scatter is larger at the tip there is still a very large difference between the two regions, in accord with Figure 5.1 (a-c). Interestingly the very end of the root region (approximately 0.2 mm) shows an increase in fluorescence intensity. Whilst this may at least partly be due to natural scatter, it could also be due to the photo-oxidation of the exposed fibre end which had occurred since shearing.

Schäfer has recently reported on the fluorescence intensities of different parts of the fibre of a Texel sheep [3,4]. Exciting at 365 nm and monitoring at 440 nm, she observed an increase in intensity of approximately four fold upon going from root to tip. The extent of weathering of wool along a fibre depends on the density of the fleece [5,6]. Hence, in a densely packed fleece, such as Merino used for Figures 5.1 and 5.3, weathering may only be observed in the extreme tips, while in an open fleece the effects of weathering is more wide spread. Examination of the coarser open fleeces from Scottish blackface sheep has shown no differences in visible fluorescence from tip to root, the whole fibre exhibiting significant intense fluorescence [2].

These results implicitly confirm the postulate in the introduction to this chapter that there exists a correlation between the occurrence of fluorescent (tryptophan) degradation products and the position along the length of a wool fibre. More recently Schäfer has provided more explicit evidence by artificially irradiating Merino wool top and observing a general decrease in tryptophan concentration together with an increase in fluorescence intensity and yellowness, as time of irradiation progressed [3,4].

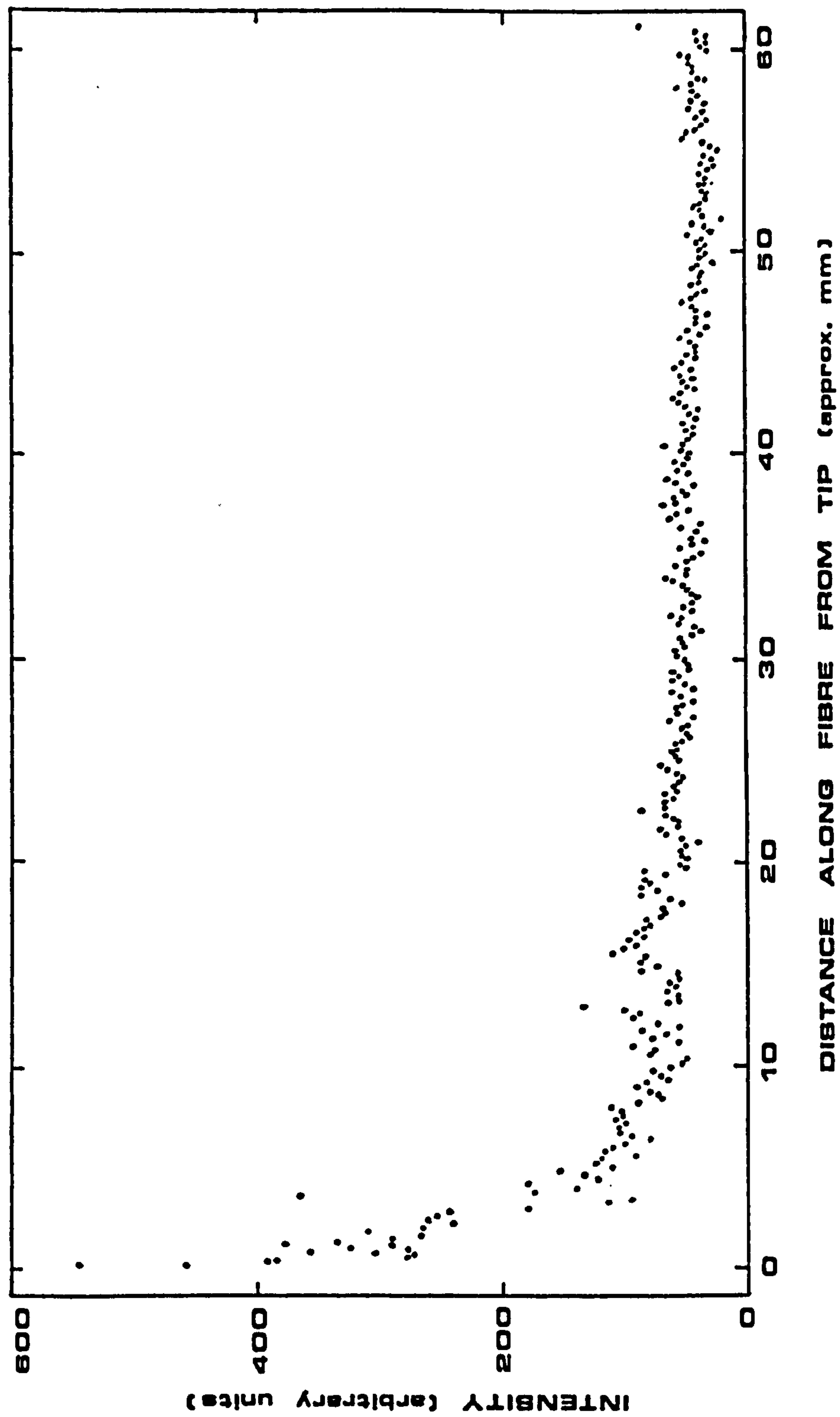


Figure 5.3 Variation in fluorescence intensity along the length of a single Merino wool fibre (488 nm excitation, fluorescence monitored at 550 nm).

5.3.2 Comparison of Fluorescent Species From Tips and Roots

The increase in fluorescence from root to tip could be due to an increase in the (same) emitting species and/or the production of a new type of fluorescent species. Figure 5.4 shows the fluorescent spectra of roots and tips of wool fibres. The spectra are similar with each having a maximum at approximately 450 nm (UV excitation). This demonstrates that the nature of the fluorescing materials in the tips and roots is similar. This was confirmed by looking at the 'fading and recovery' of the wool tips and roots.

Irradiation of wool (tips) led to 'fading' (a decrease in fluorescence intensity) i.e. the fluorescent chromophores are being destroyed by irradiation (Figure 5.5), the high rate of degradation being due to the high intensity of the light beam. If the fibre is irradiated for a short period of time e.g. 1 ms, reduction of the fluorescence intensity occurs. If this is due to chemical reduction of the chromophore, then when the light is turned off it is possible for the reduced chromophore to be oxidised by air and consequently, on re-illumination the fluorescence is of a greater intensity than when the irradiation was originally terminated. The rapidity of the recovery experienced would indicate that this is the process occurring for the fibres; such behaviour is compatible with the presence of quinone chromophores.

The rate of destruction of the natural fluorescence of the tips (Figure 5.5) and the roots (Figure 5.6) is very similar. Also, in both cases the fluorescence recovers during a dark period to similar extents. These facts suggest that the natural fluorescing species in the tips and roots are similar if not identical.

Schäfer has recently reported on the fluorescence intensities of different parts of the fibre of a Texel sheep [3,4]. Exciting with UV excitation she observed that the fluorescent emission spectra were similar at different positions along the fibre, including tip and root.

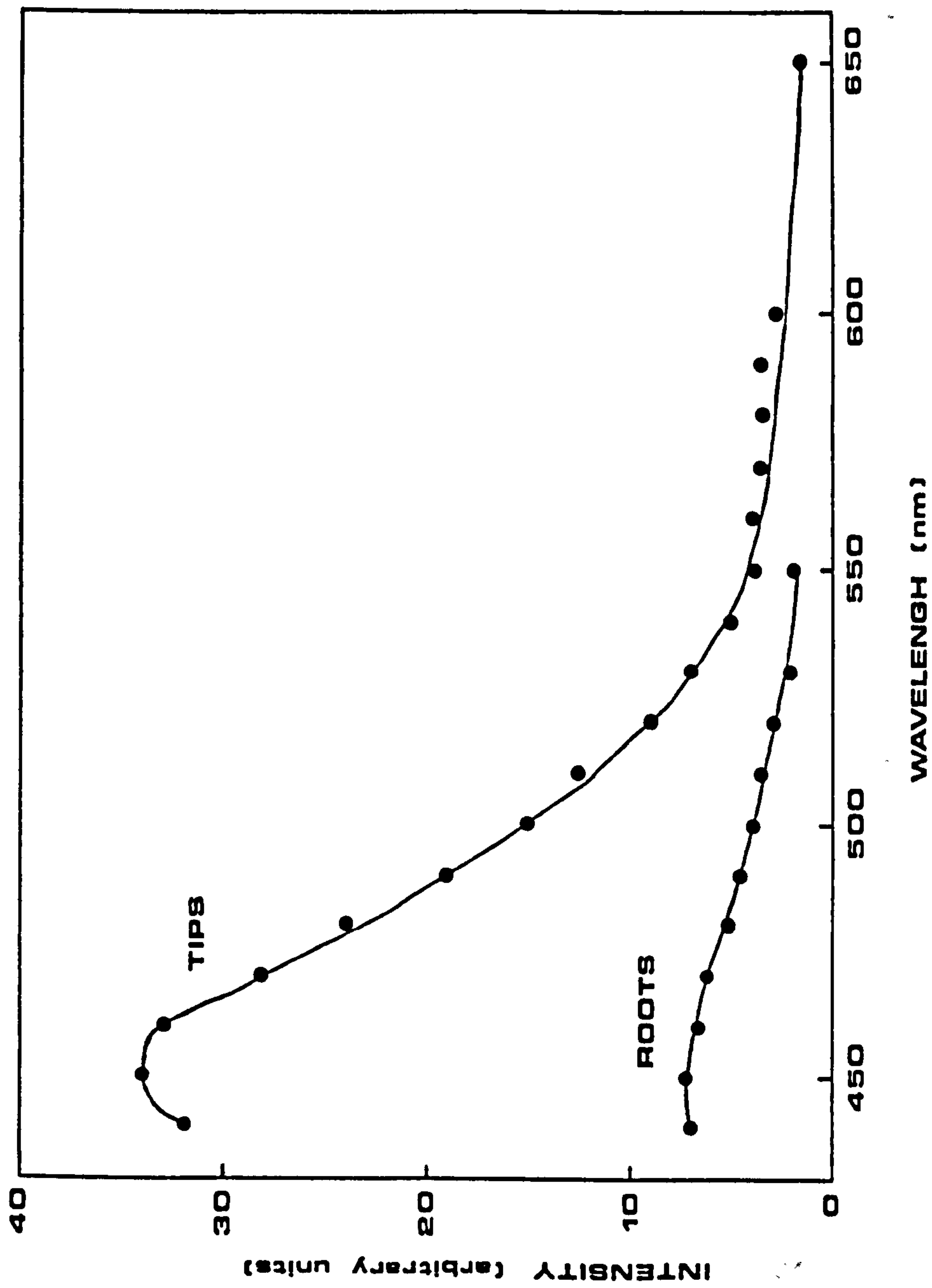


Figure 5.4 Fluorescence spectra of roots and tips of wool fibres (UV excitation).

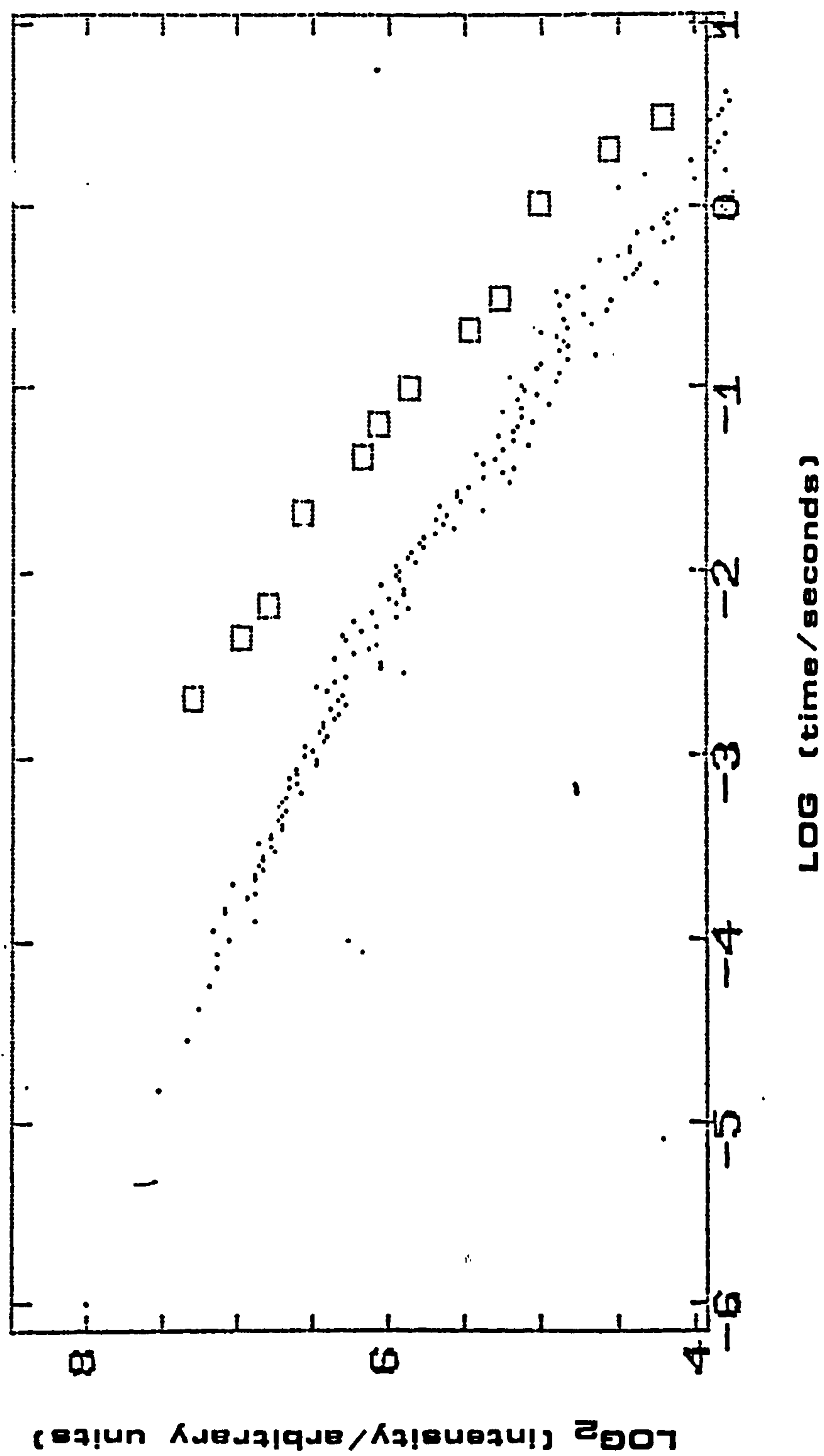


Figure 5.5 Fading of the fluorescence exhibited by wool tips (442 nm excitation, fluorescence monitored at 550 nm) showing fading (•) and recovery (□) of the signal.

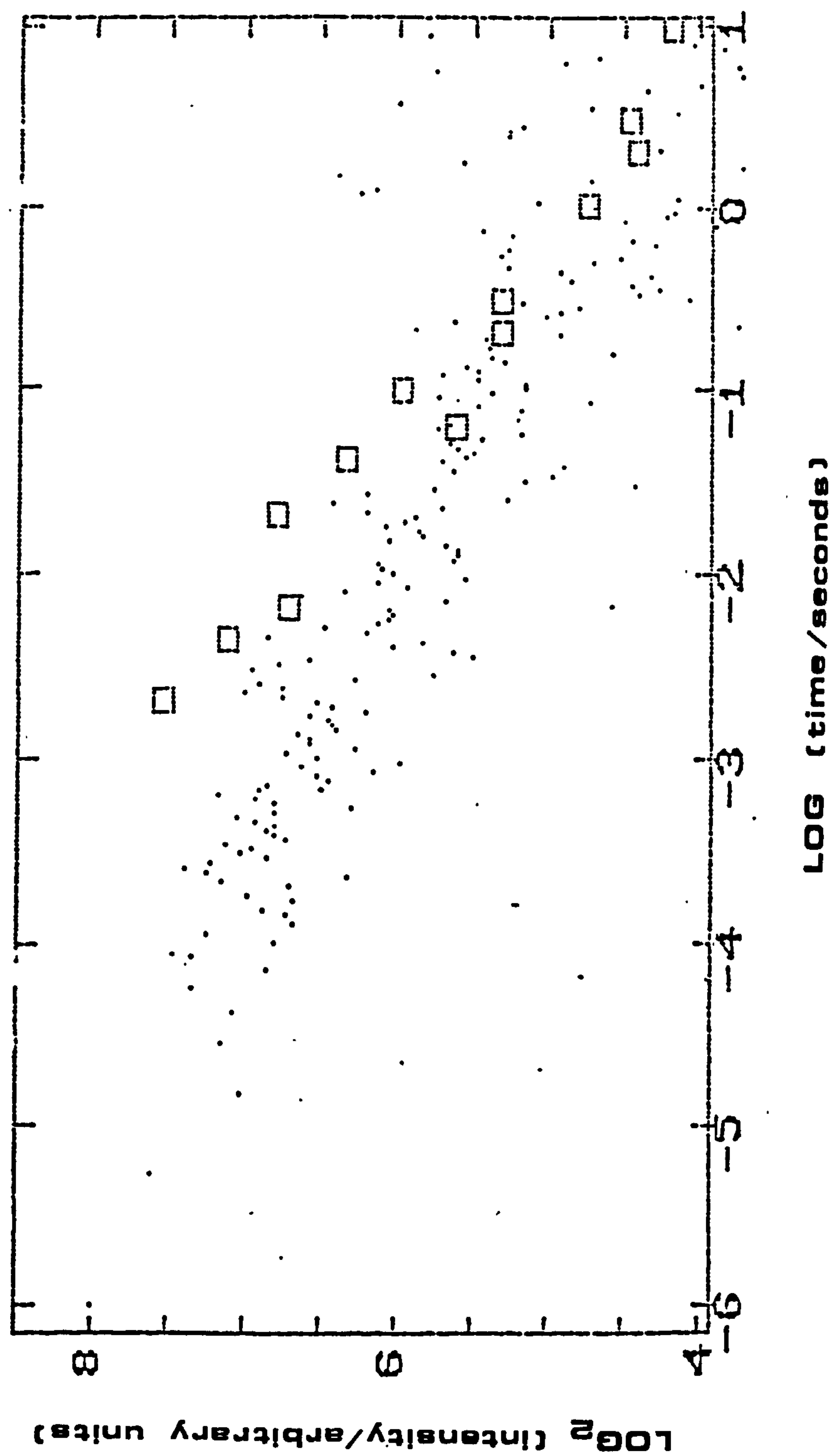


Figure 5.6 Fading of the fluorescence exhibited by wool roots (442 nm excitation, fluorescence monitored at 550 nm) showing fading (•) and recovery (□) of the signal.

Consequently, the increase in fluorescence from root to tip is due to an increase in the (same) emitting species, rather than the production of a new type of fluorescent species.

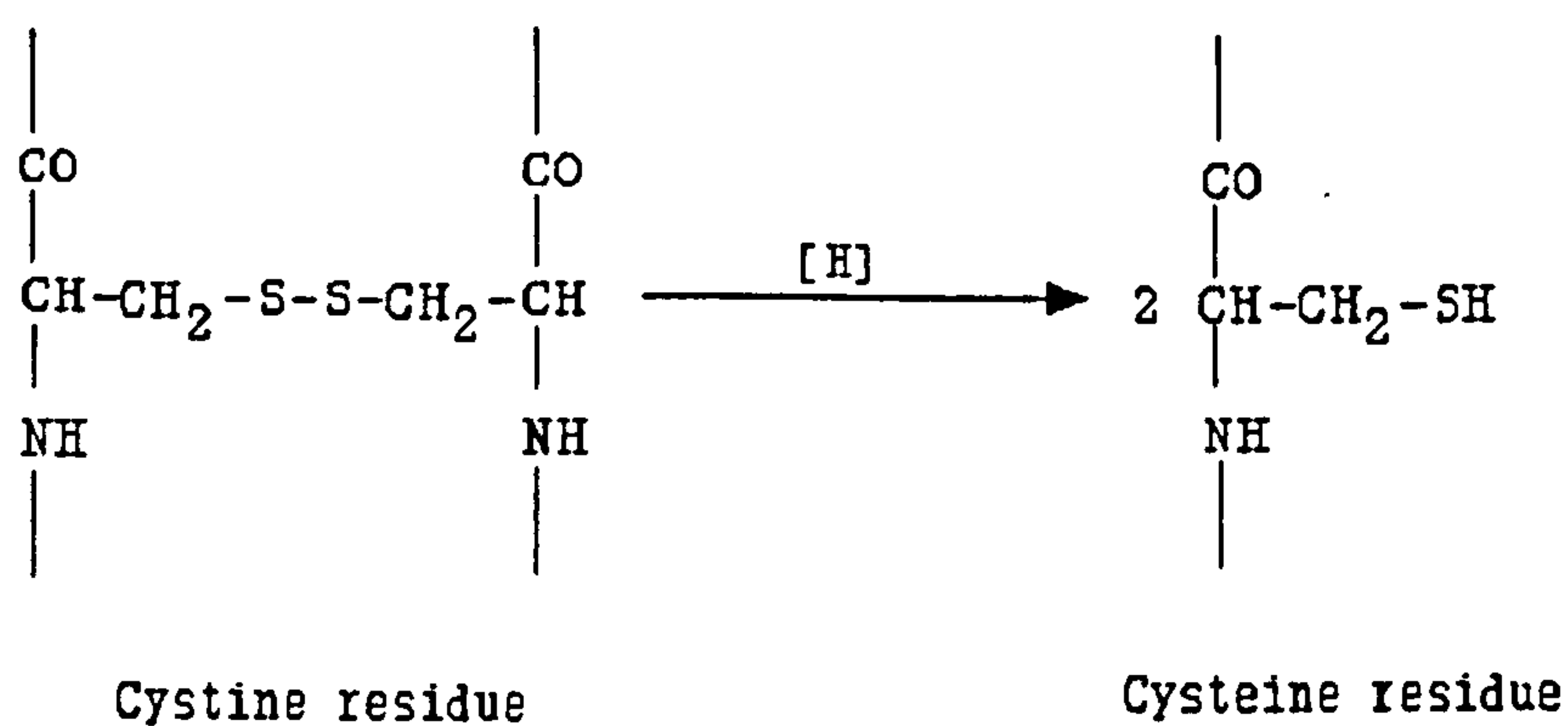
5.3.3 Effect of Disulphides

The changes in visible fluorescence along the wool fibre may be explained by the postulate that the cystine disulphide bonds in wool actively quench the fluorescence of the chromophores involved (see Section 2.5.2.2). Wool is particularly rich in cystine; in the A-layer of the exocuticle one residue in every 2.7 is half-cystine [7].

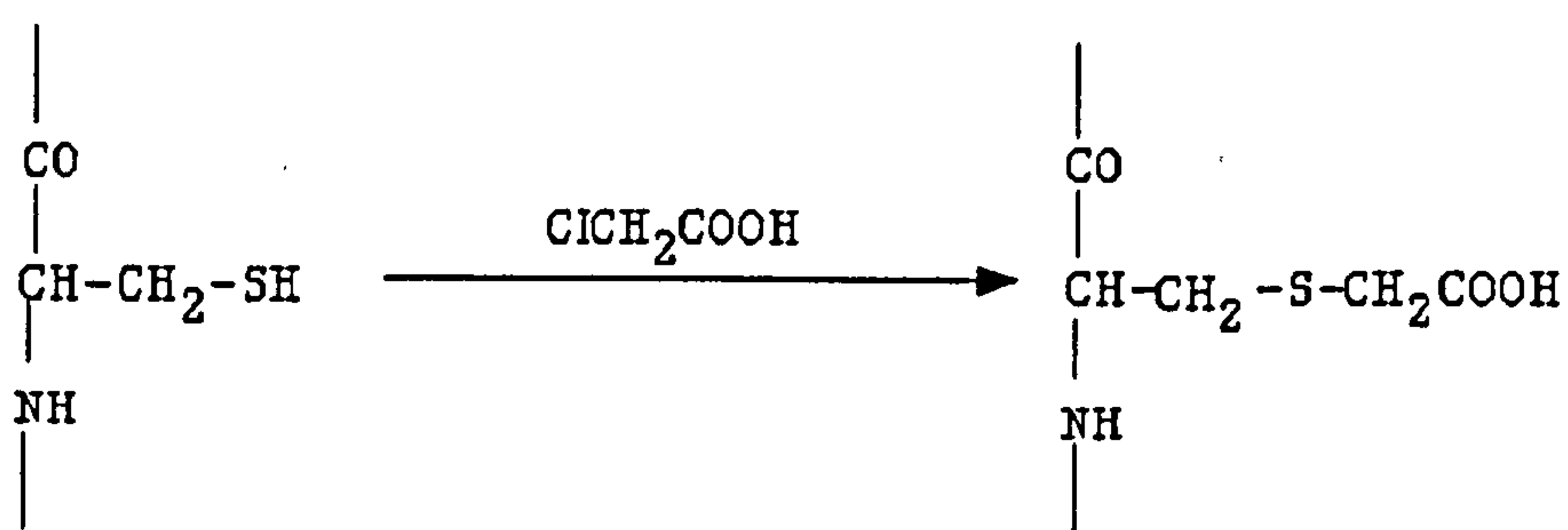
The fluorescence quenching role of the disulphides was further examined by reduction and alkylation. The reactions shown in Schemes 5.1 (reduction) and 5.2 (alkylation) were assumed to have occurred; the cysteine thiol groups formed in the initial sodium borohydride reduction being carboxymethylated with sodium monochloroacetate.

Relative to the untreated wool, the borohydride reduced wool showed an increase in fluorescence intensity of 25% and the reduced/alkylated wool an increase in fluorescence intensity of 151% (Table 5.1). This therefore underlines the important role of the disulphide bond in fluorescence quenching, since sodium borohydride reduction is not capable of forming new chromophores. The high fluorescence intensities observed following borohydride reduction indicates that the contribution of N-formylkynurenine residues to the natural visible fluorescence of wool is small (see Section 2.5.4), since this agent readily reduces carbonyl groups to alcohols.

It was also noted that the samples treated with sodium borohydride were well bleached, indicating possible applications of this agent as a reduction bleach. No obvious damage to the wool fibre was noted when treating wool with sodium borohydride under these conditions.



Scheme 5.1



Scheme 5.2

Sample of BA tops	Relative Fluorescence Intensity	
	mean	standard deviation
Untreated	100.0	12.4
Borohydride reduced	125.1	7.07
Borohydride reduced and chloroacetate alkylated	251.0	4.82

Table 5.1 Fluorescence intensities of BA wool tops after reduction/alkylation treatments.

This confirmation of the quenching role of the disulphide bonds in wool ties in with the effect of weathering (see Section 2.4.8) where photo-oxidation of (quenching) cystine residues to (non-quenching) cysteic acid residues occurs (Table 2.6). Consequently, the increase in fluorescence from root to tip is due, at least in part, to the destruction of quenching disulphide bonds.

5.3.4 Morphological Components

As already described in Section 5.3.1 cross sections of wool fibres reveal the presence of areas of high fluorescence in the form of regular dots or granules (Figures 5.1 and 5.2). These areas of high fluorescence could be randomly distributed or related to some aspect of the fibre structure e.g. a particular morphological component.

Wool was separated into the morphological components, cuticle, cortex and cell membrane complex (cmc). This was carried out using untreated wool tops as well as tops which had been bleached (oxidatively, reductively and fully). Amino acid analyses of the components were obtained and a summary of these, indicating the cysteic acid and cystine content, is shown in Table 5.2. This shows that, compared to the untreated wool tops' components, the reductively bleached tops' components generally have a variable sized increase in cysteic acid content while the oxidatively bleached tops' components have a large increase in cysteic acid content (and the fully bleached tops' components have a further small increase still). As cysteic acid is non-quenching then it is to be expected that the amount of cysteic acid will correlate with the fluorescence intensity.

Morphological Component	Amino Acid Composition (mol.100mol ⁻¹)	
	Cysteic Acid	Cystine
Cuticle		
Untreated	1.099	9.124
Oxidatively bleached	3.401	8.662
Reductively bleached	1.511	9.031
Fully bleached	2.745	8.049
Cortex		
Untreated	0.190	6.144
Oxidatively bleached	1.376	5.395
Reductively bleached	1.199	5.487
Fully bleached	1.516	5.740
CMC		
Untreated ^a	0.559	4.231
Oxidatively bleached	1.091	1.612
Reductively bleached	0.262	3.452
Fully bleached	1.338	1.962

^a the protein content of this sample was quite high and makes the result suspect.

Table 5.2 Selected amino acid compositions of morphological components.

Comparing the different components, the cortex and cmc have about the same amount of cysteic acid, while the cuticle has a larger amount. This suggests that the cuticle should be the most fluorescent. However, it is important to consider the quenching cystine. While the amount of cystine generally varies only slightly between bleaching treatments, it varies a lot between the different components. Hence, the cuticle samples which have the largest amount of non-quenching cysteic acid, also have the largest amount of quenching cystine so the two effects might 'cancel' each other out.

Infra-red spectra (not shown) of the morphological components were obtained using FTIR with a diffuse reflectance attachment. Cysteic acid is known to have a characteristic absorption at 1040-1045 cm⁻¹ [8,9]. Surprisingly, this technique was not very sensitive and so proved of little use. For example, the cysteic acid absorption peak was clearly visible from the cortex of oxidatively bleached wool but was absent from the cortex of untreated wool!

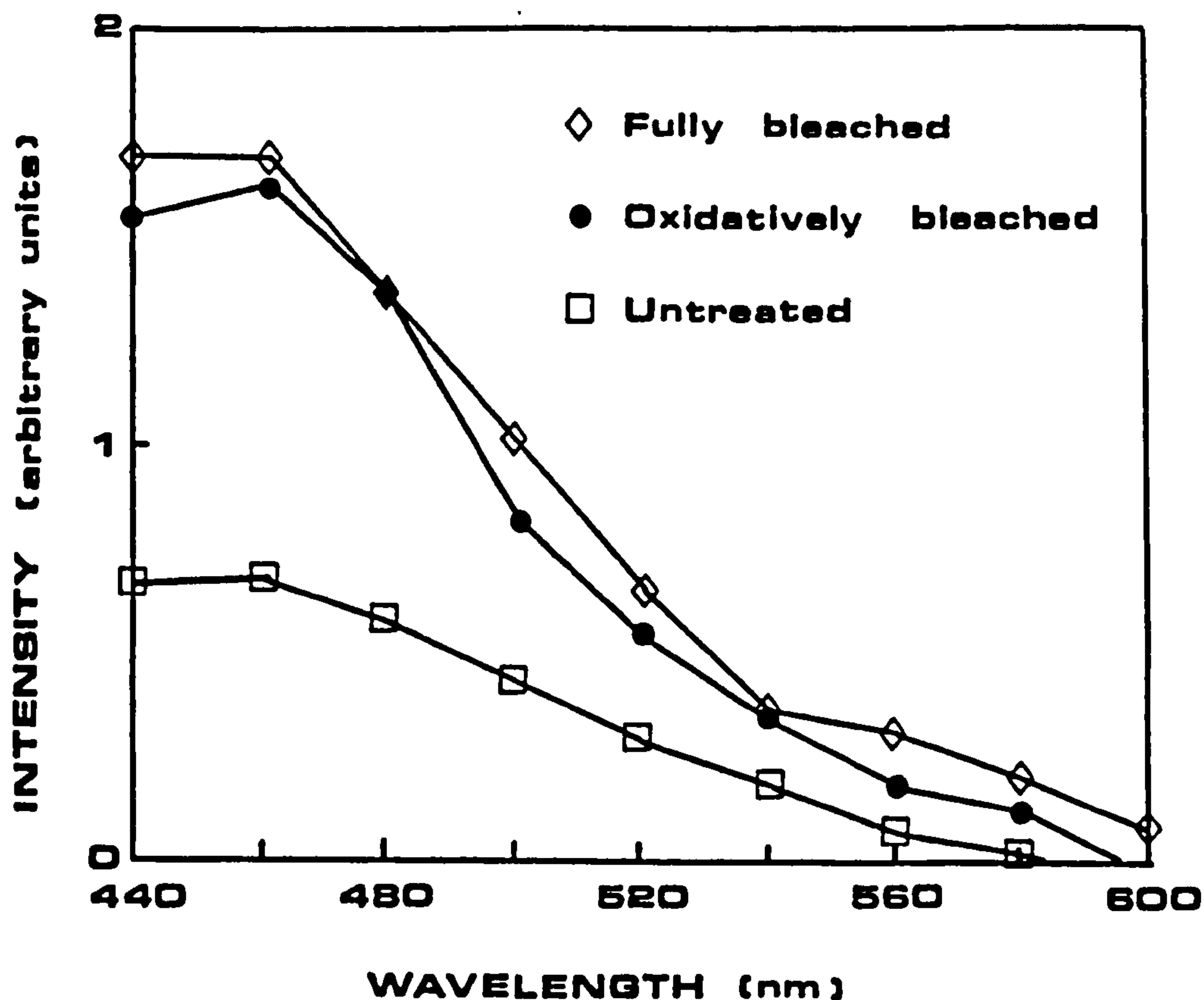


Figure 5.7 Fluorescence spectra of cortex from differently bleached wool tops (UV excitation).

Fluorescence spectra of the different morphological components were obtained using UV excitation. Figure 5.7 shows the spectra of cortex from untreated wool tops. This has a maximum at 440/460 nm and is broad. This arises from the large bandwidth used (20 nm) because of the very weak intensity of the signal. The fluorescence spectra of the cortexes from fully bleached and oxidatively bleached wool tops are similar (Figure 5.7). Indeed the fluorescence spectra of all the samples, both for the different morphological components and for the different chemical treatments used, are similar (not shown). In the measurements the signals were so weak (as well as being inhomogeneous) that it was only possible to measure spectra of samples once a brighter-than-average sample had been picked. Visual qualitative comparison suggested that the fluorescence of the bleached wool components was more intense than the untreated wool components, and that the intensity of the cortexes was greater than the cuticles or cmcs.

Morphological Component	Fluorescence Intensity							
	Untreated		Oxidatively Bleached		Reductively Bleached		Fully Bleached	
	mean ^a	s.d. ^b	mean	s.d.	mean	s.d.	mean	s.d.
Cuticle	17.3	4.97	11.2	7.68	25.7	14.68	9.7	3.62
Cortex	29.3	5.59	40.9	6.95	47.5	13.66	35.0	6.40
CMC	40.3	36.15	10.7	5.42	22.8	23.81	32.1	29.21

^a arbitrary units ^b standard deviation

Table 5.3 Fluorescence intensities of morphological components of variously bleached wools (488 nm excitation, fluorescence monitored at 550 nm).

Subsequently, an attempt was made to quantify the fluorescence intensities of the different morphological components using 488 nm laser excitation (Table 5.3). This was frustrated by difficulties in preparing uniform samples and uniformly filling the field of view in the microscope. Consequently, the results shown in Table 5.3 exhibit considerable scatter with the only meaningful deduction being that the cortex is the most fluorescent of the three morphological components. As the cuticle surrounds the cortex in the wool fibre it would be expected that the cuticle would suffer more weathering and so be more fluorescent. The increased cysteic acid content of the cuticle (Table 5.2) does indeed show that it suffers more weathering. Yet the cuticle also has a larger amount of cystine which acts as a quencher, so evidence has been provided that the proposal earlier that the two effects might 'cancel' each other out may be correct.

The use of tops for obtaining the morphological components probably caused at least some of the scatter observed in the fluorescence intensities. Tops naturally contain a wide range of fluorescent intensities (Section 5.3.1; Figure 5.2) as they contain the whole length of a wool fibre. Any effect due to different bleaching treatments or different morphological components is superimposed upon the natural

'tip-root effect'. Consequently, the effects of various treatments e.g. UV light irradiation, Section 6.3.2, were generally carried out using single wool fibres with examination being in the middle part of the fibre.

It has not been possible to correlate the areas of high fluorescence in a wool fibre cross section with any particular morphological component investigated. Further work could usefully be directed towards investigating other morphological components e.g. cortical cells or macrofibrils. However, qualitative results were obtained from the components studied: cuticle, cortex and cmc. The cuticle is the most weathered but due to the large amount of quenching cystine it contains, it is not the most fluorescent. The cortex is the most fluorescent. All three components have the same fluorescent spectra indicating the same emitting species is responsible. Bleaching treatments were found to increase fluorescence intensity (in accord with Section 2.5.2.2) and again have the same fluorescence spectra indicating the same emitting species is responsible.

5.4 CONCLUSION

The natural visible fluorescence of Merino fibres, arising from fluorescent tryptophan degradation products, varies along the length of the fibre, from highly fluorescent tips to barely fluorescent roots. The increase in fluorescence is due to an increase in the (same) emitting species, rather than the production of new types of fluorescent species, with at least some of the increase coming from the destruction of quenching cystine disulphide bonds, giving non-quenching cysteic acid residues.

Qualitative results were obtained for the morphological components: cuticle, cortex and cmc. Bleaching treatments were found to increase fluorescence intensity. Components from bleached and unbleached wools exhibited the same fluorescent emission spectra revealing the same emitting species as being responsible. The cuticle is the most weathered component but due to the large amount of cystine it contains, it is not the most fluorescent. The cortex is the most fluorescent.

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CHAPTER 6

NATURAL FLUORESCENCE OF WOOL -

EFFECT OF LIGHT IRRADIATION

6.1 INTRODUCTION

Short wavelength light (<380 nm) causes yellowing whilst blue light (>380 nm) leads to bleaching (see Section 2.4.3.2). The chemical reactions which account for these colour changes have not been identified although in the case of yellowing destruction of amino acid residues such as tryptophan, tyrosine, phenylalanine, histidine and cystine have been implicated [1]. A number of publications have drawn attention to the action spectra for yellowing and bleaching (Section 2.4.3.2 and Figures 2.7 - 2.9).

Exposure of wool to daylight leads to both bleaching and yellowing as concurrent phenomena with the resulting perceivable effect reflecting the relative extent of both processes. More extensive yellowing occurs in summer than in winter months due to the increased UV content of the light, while yellowing can be reduced by exposure behind window glass, since this reduces the UV content of light [2,3].

In Chapter 5 it was shown that the natural visible fluorescence of Merino fibres varies along the length of the fibre from highly fluorescent tips to barely fluorescent roots. This arises from the effect of weathering, including the action of sunlight (see Section 2.4.8). Sunlight contains UV and blue light. Simpson, in an article on the photobleaching of wool [4], stated that the destruction of visible absorbing chromophores may be accompanied by generation of some UV absorbing species which exhibit blue fluorescence thereby enhancing the white appearance.

This chapter describes a series of experiments which have been carried out in order to determine if UV and blue light, as well as causing different colour changes in wool, also have an effect on the natural fluorescence; and if so to establish what it is.

6.2 EXPERIMENTAL

A detailed explanation of the main materials and techniques used can be found in Chapter 4.

6.2.1 Effect of Sunlight

BA tops were exposed to sunlight for 11 weeks, August-October 1987, in Otley, Yorkshire. One sample was left in a greenhouse, while another was left outdoors. After exposure, part of the outdoor sample was cleaned using Cleaning Method 1 (shaking with tetrachloroethene). Cross sections were prepared using a Hardy microtome and photomicrographs obtained using both UV and violet excitation modes of a M17 fluorescence microscope (Section 4.4.2.1). Camera exposure times of 30 seconds (Kodachrome 64 ASA (Daylight) colour transparency film) were found to produce good images.

6.2.2 Effect of UV Irradiation

Wool staples from Sharlea fleece 2 were placed on a piece of filter paper and irradiated using Irradiation Method 1 equipped with UV lamps (Thorn, 3 x 20W, 600 mm, peak emission 365 nm). Staples which had been previously cleaned ('clean') (using Cleaning Method 3) and those which had not ('dirty') were irradiated. It was noticeable that the wool (more so the clean than the dirty) became dirty as time progressed, presumably due to air pollution. Also it was observed that the wax became harder on the dirty staples. During irradiation the staples were periodically turned over. After irradiation for the required period of time the staples were (re)cleaned using Cleaning Method 3.

Small portions of fibres were embedded on a glass slide using glycerol. The portions came from the middle part of the staple so as to exclude the effect of natural weathering. The fibre portions were examined using a LAB16 fluorescence microscope equipped with a Neofluar 16/0.40 objective (Section 4.4.2.3). UV excitation mode was used and all the fluorescence emission produced was monitored. 15 measurements were made for each sample. The colour of the samples was determined visually.

6.2.3 Effect of Blue (Northlight) Irradiation

Three experiments using blue light irradiation were performed:

- (i) Pieces of fabric (20 x 5 cm, supported on rectangular glass plates of similar dimensions) were irradiated using Irradiation Method 1 equipped with Northlight lamps (Thorn, 3 x 20W, 600 mm). Before irradiation the samples were dipped in aqueous Lissapol N and rinsed. During the irradiation the samples were immersed under 1-2 cm of water with a Perspex sheet (6 mm) immediately on top of the fabrics to act as a UV filter. This did not prohibit movement of water around the samples. Each sample was divided into two halves. One half was covered with aluminium foil whilst the other half was exposed for the required period of time. After irradiation the samples were rinsed in cold water and left to dry at room temperature in the dark.

The fluorescence intensities of the wool fabrics were obtained using a MPF-4 fluorescence spectrophotometer (Section 4.4.1). The samples were excited at 350 nm and the fluorescence emission measured between 360 and 640 nm. For each of the samples three portions were measured. Yellowness Index values of the fabrics were also determined.

- (ii) Wool staples from Merino fleece 2 were placed on a piece of filter paper and irradiated using Irradiation Method 1 equipped with Northlight lamps (Thorn, 3 x 20W, 600mm). A Perspex sheet (6mm) was used as a UV filter but this did not prevent air movement around the staples. Staples which had been previously cleaned ('clean') (using Cleaning Method 3) and those which had not ('dirty') were irradiated. During irradiation the staples were periodically turned over. After irradiation for the required period of time the staples were (re)cleaned using Cleaning Method 3.

Small portions of fibres were embedded on a glass slide using glycerol. The portions came from the middle part of the staple so as to exclude the effect of natural weathering. The fibre portions were examined using a IM fluorescence microscope equipped with a Neofluar 16/0.40 objective (Section 4.4.2.4). UV excitation mode was used and all the fluorescence emission produced was monitored. The intensities were measured twice, first with 15 measurements for each sample and then with 40.

- (iii) Wool staples from Sharlea fleece 1 were irradiated the same as for Merino fleece 2 above, except that laminated glass (6mm) was used as the UV filter instead of the Perspex sheet. The fluorescence intensity measurements were determined the same as for Merino fleece 2 above, except that only one set of measurements involving 40 measurements for each sample, was made.

6.2.4 Laser Irradiation

Two experiments using laser irradiation were performed:

- (i) Preliminary investigations were carried out using either a 50 mW 442 nm helium cadmium laser (Irradiation Method 2) and/or UV lamps (Irradiation Method 1). Pieces of fabric were used.

When Irradiation Method 2 was used the fabric was either irradiated wet or dry. If the fabric was wet then it had first been dipped in aqueous Lissapol N and then rinsed. After irradiation the fabric was left to dry at room temperature in the dark.

When Irradiation Method 1 was used the fabric was irradiated wet. Before irradiation the samples were dipped in aqueous Lissapol N and rinsed. During the irradiation with UV lamps (Thorn, 3 x 20W, 600 mm, peak emission 365 nm), the samples were immersed under 1-2 cm of water.

One half of each sample (not containing the laser irradiated portion if applicable) was covered with aluminium foil whilst the other half was exposed for the required period of time. After irradiation the samples were rinsed in cold water and left to dry at room temperature in the dark.

The colour of the samples was determined visually.

- (ii) Quantitative investigations were carried out using fabric with a 15W Argon/Krypton laser (Irradiation Method 3). Yellowness Index values and diffuse reflectance spectra were obtained for some samples. The fluorescence intensities of the samples was initially obtained using a MPF-4 fluorescence spectrophotometer (Section 4.4.1). The samples were excited at 360 nm and the fluorescence emission measured between 370 and 620 nm.

Subsequently, portions of the samples were examined using a IM fluorescence microscope equipped with a F-Achromat 32/0.4 objective (Section 4.4.2.4). Visible excitation mode was used and all the fluorescence emission produced was monitored. The portions of the fabrics from the centre spot (5mm diameter) were used, after being embedded on a glass slide using glycerol. 50 measurements were made for each sample.

6.2.5 Other Investigations

- (i) Comparison of treatments. Untreated fabric, fabric which had been oxidatively bleached (Bleaching Treatment 1), fabric which had been irradiated using a 50 mW 442 nm helium cadmium laser for 390 min (Irradiation Method 2), and fabric, embedded on a glass slide using immersion oil, which had been irradiated *in situ* on the LAB16 fluorescence microscope, equipped with a 5/0.15 objective, for 5 min using UV excitation mode (Irradiation Method 4), were used. Small pieces of the fabrics were embedded on a glass slide using immersion oil. The fabrics were examined using a LAB16 fluorescence microscope equipped with a 5/0.15 objective (Section 4.4.2.3). UV excitation mode was used

and the fluorescence intensity at various wavelengths measured. The results are the average of three readings, except for the *in situ* irradiation with UV excitation, which was carried out only once with the fluorescence being measured straight after the irradiation. The results are corrected for the decrease of the fluorescence intensity which occurred during the period of measurement.

- (ii) Effect of embedding medium. Pieces of fabric (6 cm x 7.5 cm, held onto glass plates of similar dimensions by aluminium foil) were irradiated using Irradiation Method 1 equipped with Blacklight lamps (Philips, 3 x 18W, 600 mm, emitting UV) for three days. During irradiation the fabrics were covered by a solvent - either water or immersion oil. Before irradiation the water sample was dipped in aqueous Lissapol N and rinsed, while the immersion oil sample was squeezed in immersion oil. After irradiation the water sample was rinsed in water, while the immersion oil sample was soxhlet extracted using toluene (AnalaR, BDH) followed by rinsing in n-pentane (Rathburn, hplc grade) and thorough rinsing in water (similar to Cleaning Method 3). Both were air dried in the dark. The irradiation was carried out in duplicate. In the second case ordinary glass (5 mm) was used as a filter (Figure 4.2) but this was placed so that it did not prevent air moving between the top of the solvent container and the bottom of the filter.

The fluorescence intensities of the wool fabrics were obtained using a MPF-4 fluorescence spectrophotometer (Section 4.4.1). The samples were excited at 360 nm and the fluorescence emission measured between 370 and 610 nm. For each of the fabric samples six portions were measured. Yellowness Index values of the fabrics were also determined.

6.3 RESULTS AND DISCUSSION

6.3.1 Effect of Sunlight

The effect of sunlight has already been observed in passing in Section 5.3.1. There it was observed that the natural visible fluorescence of Merino fibres varies along the length of the fibre from highly fluorescent tips to barely fluorescent roots. This variation in fluorescence arose not only from the effect of sunlight (which is a mixture of light of different wavelengths) but also from variation in temperature, humidity etc. and so, should really be ascribed to the cumulative effect of natural weathering '*in utero*'. In this section the effect of sunlight (natural weathering) '*in vitro*' is briefly investigated, before later sections deal with the effect of light of different wavelengths in more detail.

BA tops were exposed to sunlight for three months, either in a greenhouse or outdoors, and fluorescence micrographs obtained (not shown). UV and violet excitation modes produced micrographs of similar intensity. The micrograph of untreated BA tops showed a distribution of highly fluorescent tips right through to virtually non-fluorescent roots, with a spotty pattern in the cortex of some fibres being visible (c.f. Section 5.3.1 and Figure 5.2). Compared to this tops which had been exposed outdoors showed a uniform level of fluorescence intensity with an increase in the median level but with hardly any 'spotty cortexes' and having an absence of very bright fibres (cleaning the outdoor sample before examining it had no effect). The micrograph of the tops which had been exposed in a greenhouse was similar to the micrograph of the outdoor sample, except that there were many more 'spotty cortexes'. The difference between the effect of sunlight (increasing fluorescence but no bright fibres) and the sum of the effects of UV irradiation (Section 6.3.2, increasing fluorescence) and blue light irradiation (Section 6.3.3, no change in fluorescence) is unclear but may be due to the other components of weathering e.g. atmospheric dust, humidity, which were not investigated.

Consequently, irradiation with sunlight causes the difference in root-tip visible fluorescence to disappear with the median level of fluorescence increasing. Using a greenhouse, whose glass (c.f. Figure 4.2) would reduce the amount of the sun's UV rays falling on the wool, had only a minor effect.

6.3.2 Effect of UV Irradiation

The effect of the UV component of sunlight on the natural fluorescence of wool was investigated by irradiating Sharlea type fibres with UV light. There is a large increase in fluorescence intensity upon irradiation (Figure 6.1) and photoyellowing slowly occurs. By irradiating both 'clean' and 'dirty' wool fibres (and measuring after subsequent cleaning) it is observed that although the fluorescence of both increase, the dirty fibres do so at a much slower rate. Over a period of 700 hours the intensity of the clean wool increased by 160% and the dirty by 110%.

Schäfer has recently reported on the effect of UV irradiation (Xenotest 150 using 4 IR/3 glass filters with a black panel temperature of 80-85°C) upon the natural fluorescence of Merino wool tops [5,6]. She found a large increase in fluorescence intensity upon irradiation - over a period of 500 hrs the intensity increased over 11 fold! The apparent difference in the size of the increase between her result and that shown by Figure 6.1 may be partly explained by the fact that she first cleaned the tops using methylene chloride, as chlorinated solvent retention promotes photoyellowing [7]. She also observed that heating at 115°C caused the fluorescence of wool top to increase (approximately three fold over 5 days) [6]. Chemical destruction of tryptophan occurs above 100°C [8]. If the Xenotest had allowed the temperature to vary a lot, then this could have contributed to the increase in fluorescence intensity observed upon irradiation.

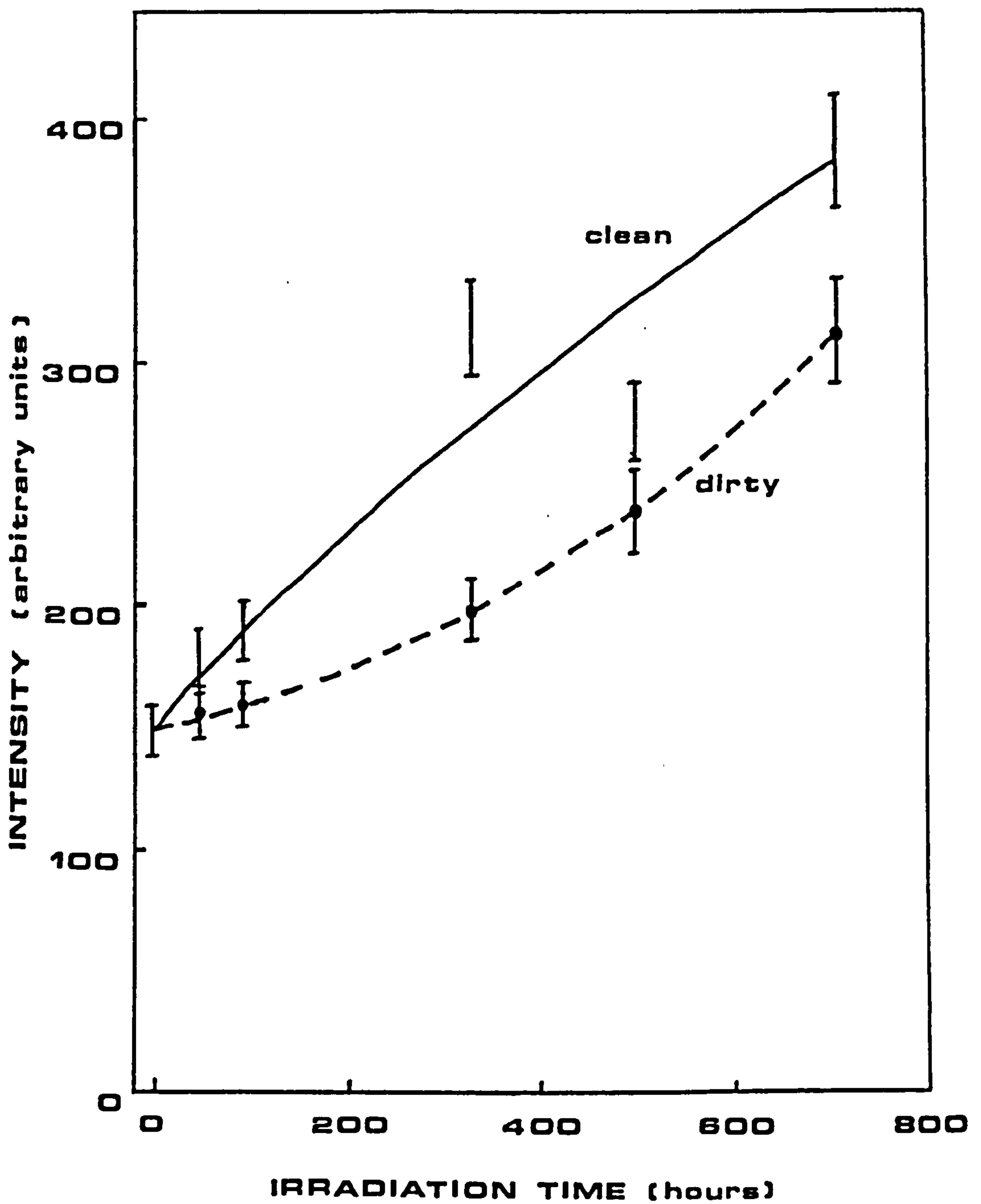


Figure 6.1 Variation in fluorescence intensity with time of UV irradiation for Sharlea type fibres (UV excitation, $n=15$, mean \pm standard error of the mean).

It is known that suint has a protective effect against photoyellowing with the effect of the natural level of wax being unclear (Section 3.2.3.2). The finding (Figure 6.1) that 'dirty' wool experiences a smaller increase in fluorescence intensity upon irradiation is interesting. It is obvious that the wool yolk is having a protective effect although it is not clear which component(s) is causing this. It is not due to the density of the fleece excluding light as the wool staples were separated slightly from one another before irradiation although the wool fibres inside the staples were left together (the same arrangement was used for the clean staples).

Hence, UV irradiation is seen to produce a rapid increase in natural fluorescence of wool. This is in accord with the observation that UV irradiation causes photoyellowing (Section 2.4.3.2) and more fluorescent degradation products (Section 5.3.1). At the same time wool yolk exhibits a definite protective effect.

6.3.3 Effect of Blue (Northlight) Irradiation

The effect of the blue component of sunlight on the natural fluorescence of wool was investigated using Northlight fluorescent tubes. These emit mainly in the visible with an emphasis on blue light and have a small amount of UV (Figure 4.1). To eliminate the UV component a filter was used.

Fabrics were irradiated in the wet state using Perspex as the filter (Figure 4.2). As the time of irradiation increased the samples became progressively whiter (Table 6.1). This is in accord with the known phenomenon of photobleaching (Section 2.4.3.2). However, statistical analysis (t-test) showed that there was no significant difference ($P=0.05$) in fluorescence intensity upon irradiation.

Irradiation Time (hour)	Yellowness Index	Fluorescence mean ^a	Intensity s.d. ^b
0	22.30	100.0	4.8
27	18.68	100.1	7.5
50	17.72	92.5	11.7
147	17.26	112.8	13.4
340	16.41	98.3	4.9

^a arbitrary units ^b standard deviation

Table 6.1 Yellowness Index values and relative fluorescence intensities (350 nm excitation) of fabric after Northlight irradiation.

Merino staples were also investigated with Northlight irradiation using Perspex as the filter. Both clean and dirty samples were irradiated with fluorescent measurements obtained after subsequent cleaning (Figure 6.2). Both show a variation in fluorescence intensity as the irradiation time increases. However, this effect seems to be random as there is no observable trend. In an attempt to increase the precision of the fluorescence intensities the measurements were repeated using a larger number of portions (40 instead of 15) (Figure 6.3). Although the error bars are a lot smaller, the same (but smaller) random variation is observed.

To be certain that the small amount of UV emitted by the Northlight lamps was not interfering, the Perspex filter was replaced by laminated glass and the irradiation experiment was then repeated using Sharlea wool. Both clean and dirty samples were investigated as before and a large number of measurements made (40) (Figure 6.4). A random variation, similar to the earlier measurements, was observed.

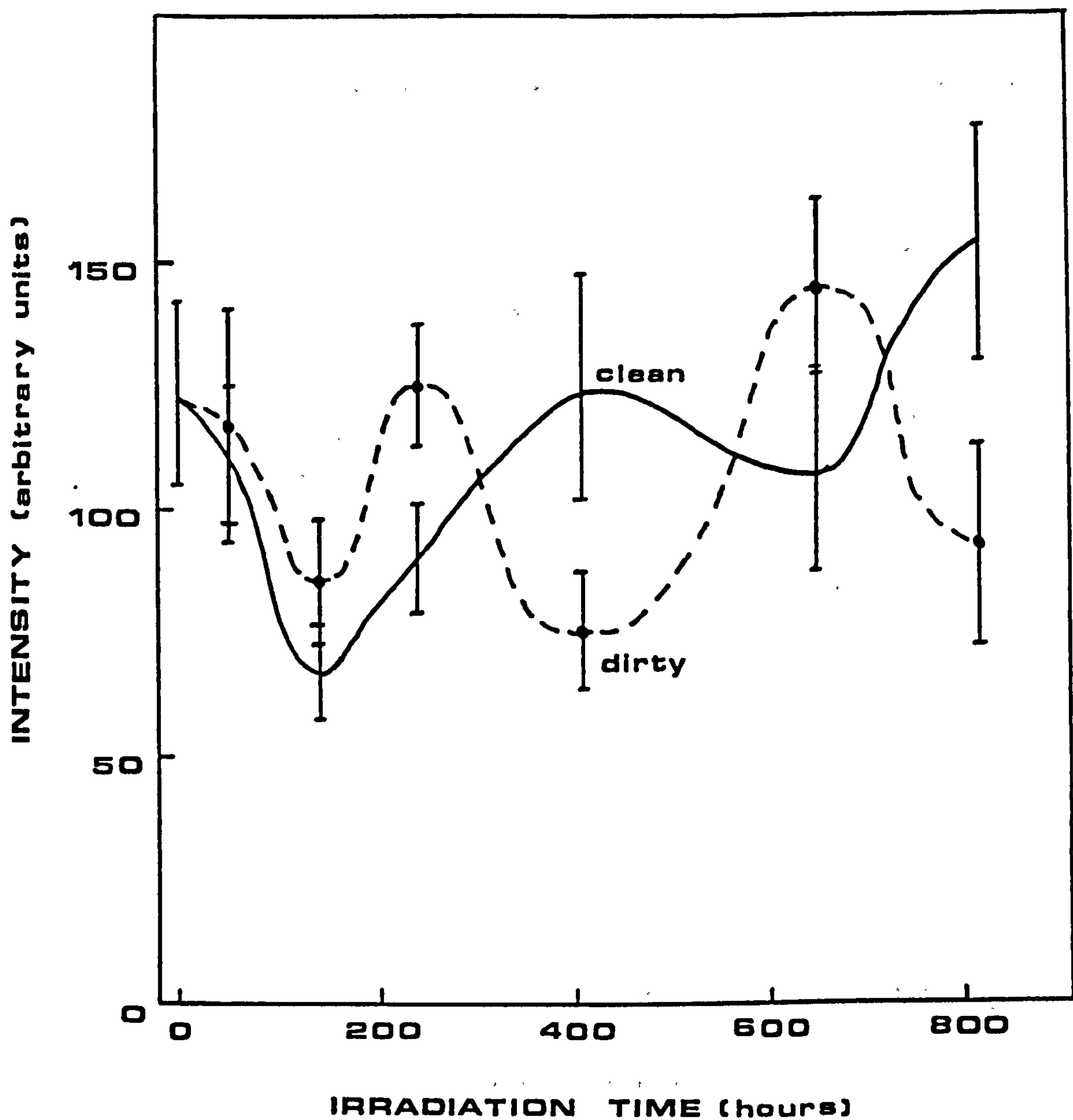


Figure 6.2 Variation in fluorescence intensity with time of Northlight irradiation (Perspex filter) for Merino fibres (UV excitation, $n=15$, mean \pm standard error of the mean).

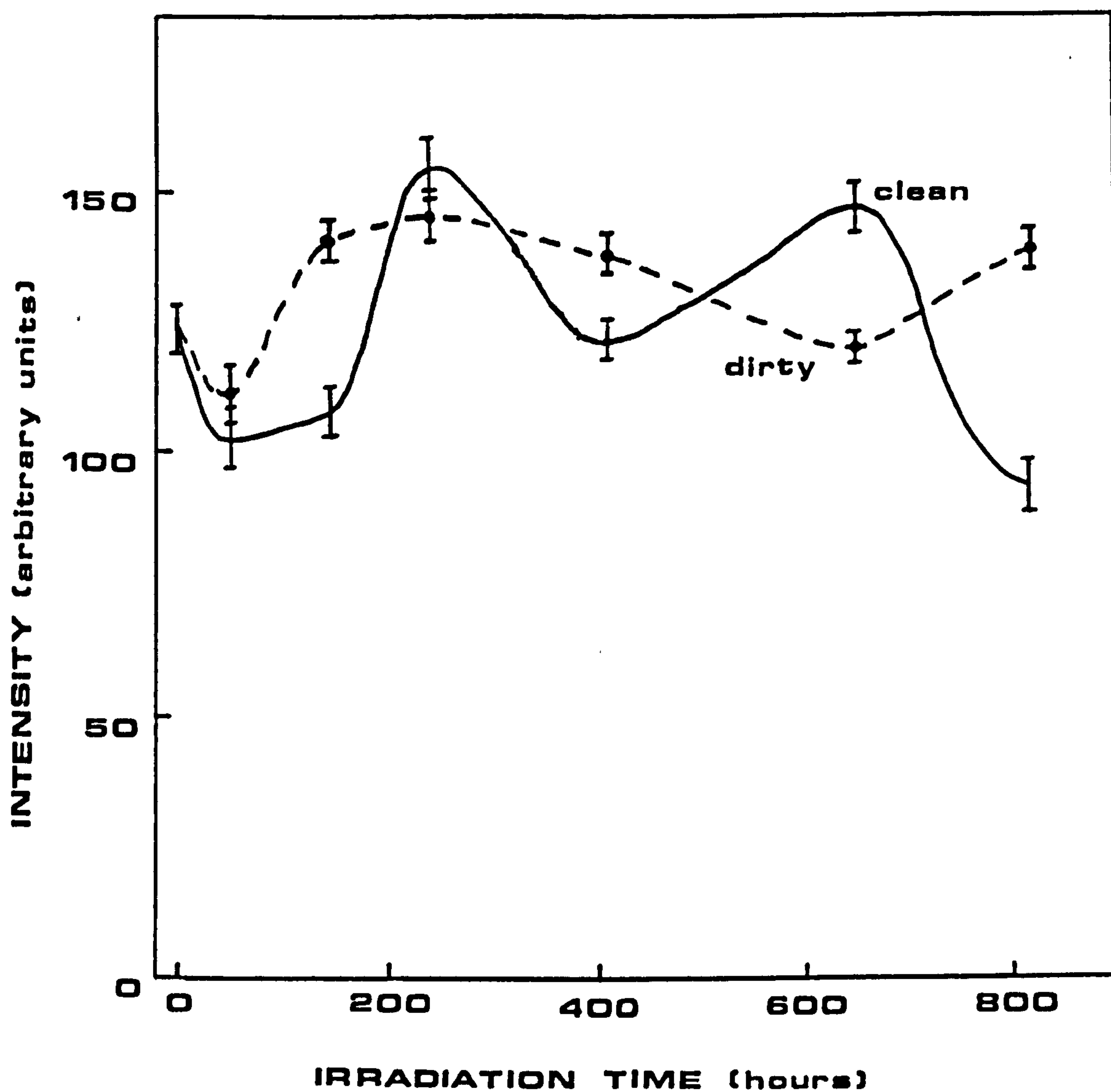


Figure 6.3 Variation in fluorescence intensity with time of Northlight irradiation (Perspex filter) for Merino fibres (UV excitation, $n=40$, mean \pm standard error of the mean).

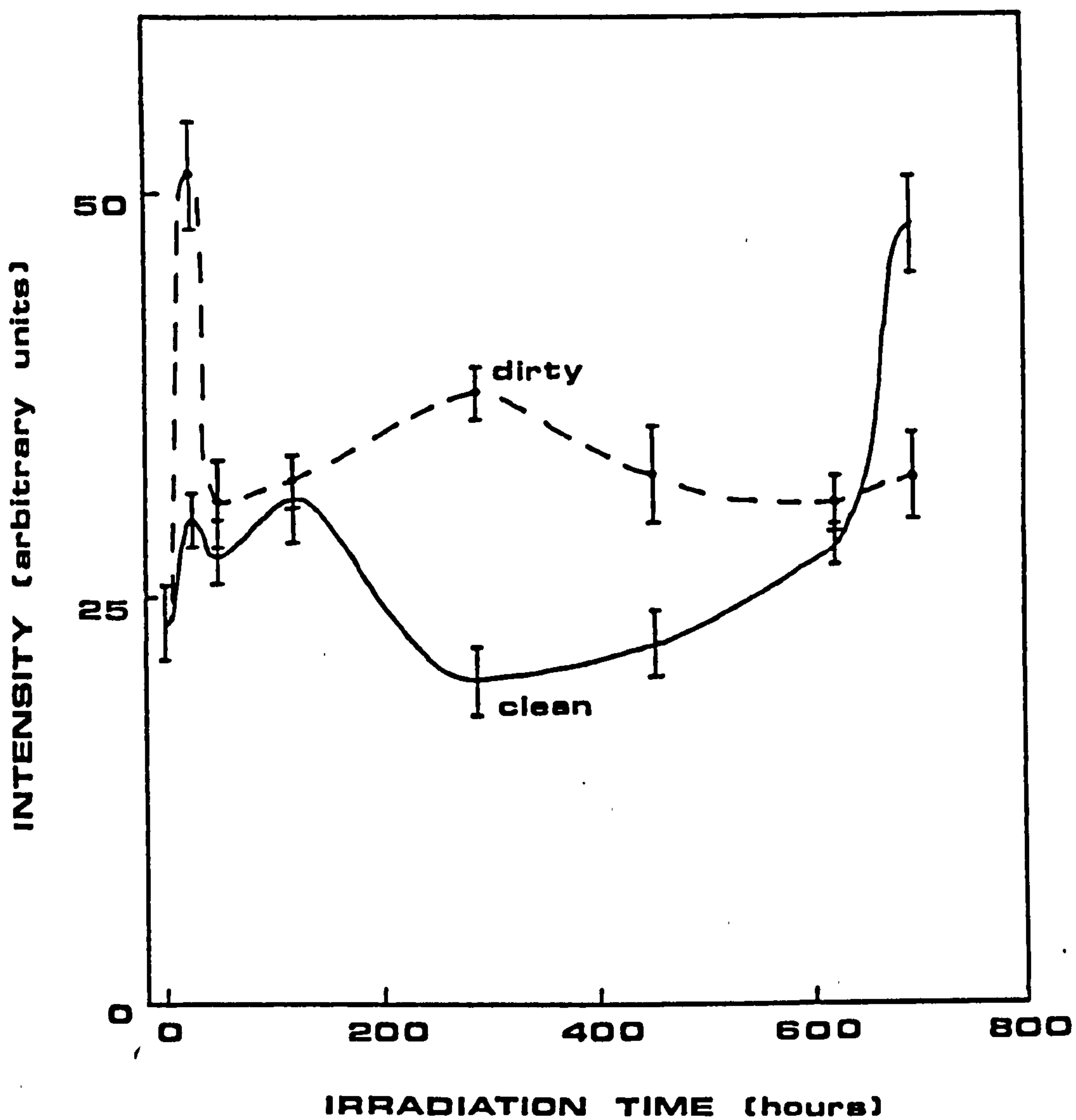


Figure 6.4 Variation in fluorescence intensity with time of Northlight irradiation (laminated glass filter) for Sharlea fibres (UV excitation, $n=40$, mean \pm standard error of the mean).

These three experiments (at ambient temperature) each show that blue light does not have any effect on the natural fluorescence of wool; with any variation upon irradiation being within a normal distribution. Hence, the enhancement of whiteness observed with photobleaching is not due to an increase in the amount of fluorescence exhibited as suggested by Simpson [4]. Instead of sensitising oxidation of disulphide bonds (as happens in peroxide bleaching), the blue light may be triggering off destruction of yellow coloured residues, possibly via an oxidative mechanism.

Schäfer has recently reported on the fluorescence of wool during 'photobleaching' [6]. During photobleaching of initially yellow wool a decrease in fluorescence was observed which reversed upon longer exposure. The apparent difference in the effect of photobleaching between Schäfer's results and those shown by Figures 6.2-6.4 together with Table 6.1 is due, at least partly, to the use of impure blue light sources. Schäfer used daylight behind window glass and an Atlas ES25 Weatherometer (averaged daylight outdoors), both of which permit some UV to fall onto the wool. The window glass would filter out some UV but not all (Figure 4.2). When wool is exposed to polychromatic light, notably sunlight, photoyellowing and photobleaching proceed simultaneously, the overall effect being determined partly by the relative intensities of the UV and blue light regions of the spectrum as well as the exposure period and initial natural yellowness of the sample [9]. Hence, Lennox and King [9] observed photobleaching and then photoyellowing with wool samples after irradiation with a Xenotest. Consequently, Schäfer's reports on the fluorescence of wool during photobleaching are ambiguous because polychromatic light sources were used, although the effect of different yellow coloured wools is commented upon. This aspect, using pure blue light sources, is something which could profitably be explored in the future.

There is no apparent difference between the fluorescence intensities of the clean and dirty wool staples (Figures 6.2-6.4). As blue light has no effect on the fluorescence intensity of clean wool, then it is hardly surprising that the wool yolk does not have a protective effect. This result does show that the wool yolk is not acting as a photosensitiser, since if this were the case it would enable the wool fibre to be damaged by light of a wavelength which would not otherwise harm it.

Hence, irradiation with blue light (photobleaching) does not produce any change in the natural fluorescence of wool and so the increase in whiteness observed during photobleaching cannot be due to the production of new fluorescent species but rather to the destruction of yellow coloured residues. Additionally, wool yolk has no apparent protective effect upon wool subjected to blue light.

6.3.4 Laser Irradiation

As described in the previous section, when wool is exposed to mixed radiation, notably sunlight, photoyellowing and photobleaching proceed simultaneously [9]. The previous two sections have described the effect of UV (Section 6.3.2) and blue light (Section 6.3.3) upon the natural fluorescence of wool. To be doubly certain that the results obtained were not compromised by employing impure light sources a 15W argon ion laser providing outputs at 488 or 351 nm was used to further investigate the effect of light irradiation on the natural fluorescence of wool. The characteristics of laser irradiation include its coherence, non-divergence and (especially in this application) its monochromaticity [10].

First preliminary experiments were carried out using a helium cadmium laser (50mW, 442 nm) for supplying blue light and UV tubes for supplying UV light. Fabric was irradiated with 442 nm blue light. As the time increased the samples became progressively whiter (Table 6.2).

Irradiation Time (minutes) (dry unless stated)	Colour of Fabric
15	no visible change
120	weak white spot
345	white spot
390	definite white spot
495	'bright white spot'
60 wet	weak white spot

Table 6.2 Variation in colour of fabric with time of 442 nm laser irradiation (50 mW, 5 mm diameter)

This is in accord with the known phenomenon of photobleaching (Section 2.4.3.2). Examination of the fluorescence of the 390 min irradiated spot (UV excitation; Section 6.3.5) revealed it to be less fluorescent than untreated fabric. The multiple effect of UV and blue light was also looked at. Irradiation of untreated fabric with UV for 93½ hours turned the fabric yellow; subsequent irradiation with 442 nm laser for 480 minutes (8 hours) produced a white spot. Irradiation of untreated fabric with 442 nm laser for 495 min produced a white spot (Table 6.2); subsequent irradiation with UV for 65 hours caused the fabric to go yellow with no spot visible. So, at least as far as the colour is concerned, photobleaching and photoyellowing seem reversible.

Secondly, quantitative investigations were carried out using a 15W argon ion laser (488 or 351 nm). Fabrics, dry (normal regain) and wet, were irradiated for either 1 or 4 hours. Diffuse reflectance spectra obtained from the samples irradiated for 4 hours are shown in Figure 6.5 (dry) and Figure 6.6 (wet).

UV irradiation (351 nm) of the dry fabric led to yellowing, there being a small increase in visible absorbing chromophores as well as a large increase in UV absorbing chromophores. The blue irradiation (488 nm) however, caused a decrease in visible absorbing chromophores (causing the wool to appear whiter), and at the same time producing a small increase in UV absorbing chromophores.

Similar effects were observed on irradiation of wet fabric (Figure 6.6) except that the magnitude of the changes was larger. It is known that wet fabrics yellow much faster than dry fabrics [11], and this is confirmed by the Yellowness Index (YI) values (Table 6.3). For wet wool UV irradiation caused an increase in YI value from 23.87 to 39.32 whilst blue light caused a decrease to 16.98. For this quite short irradiation period (4 hours) these are quite large changes. The YI values relating to irradiation of dry wool are interesting. Despite the UV irradiated sample having a yellow appearance its YI value is lower than the unirradiated sample. By way of contrast the sample irradiated with blue light had an even lower YI value than the wet irradiated sample.

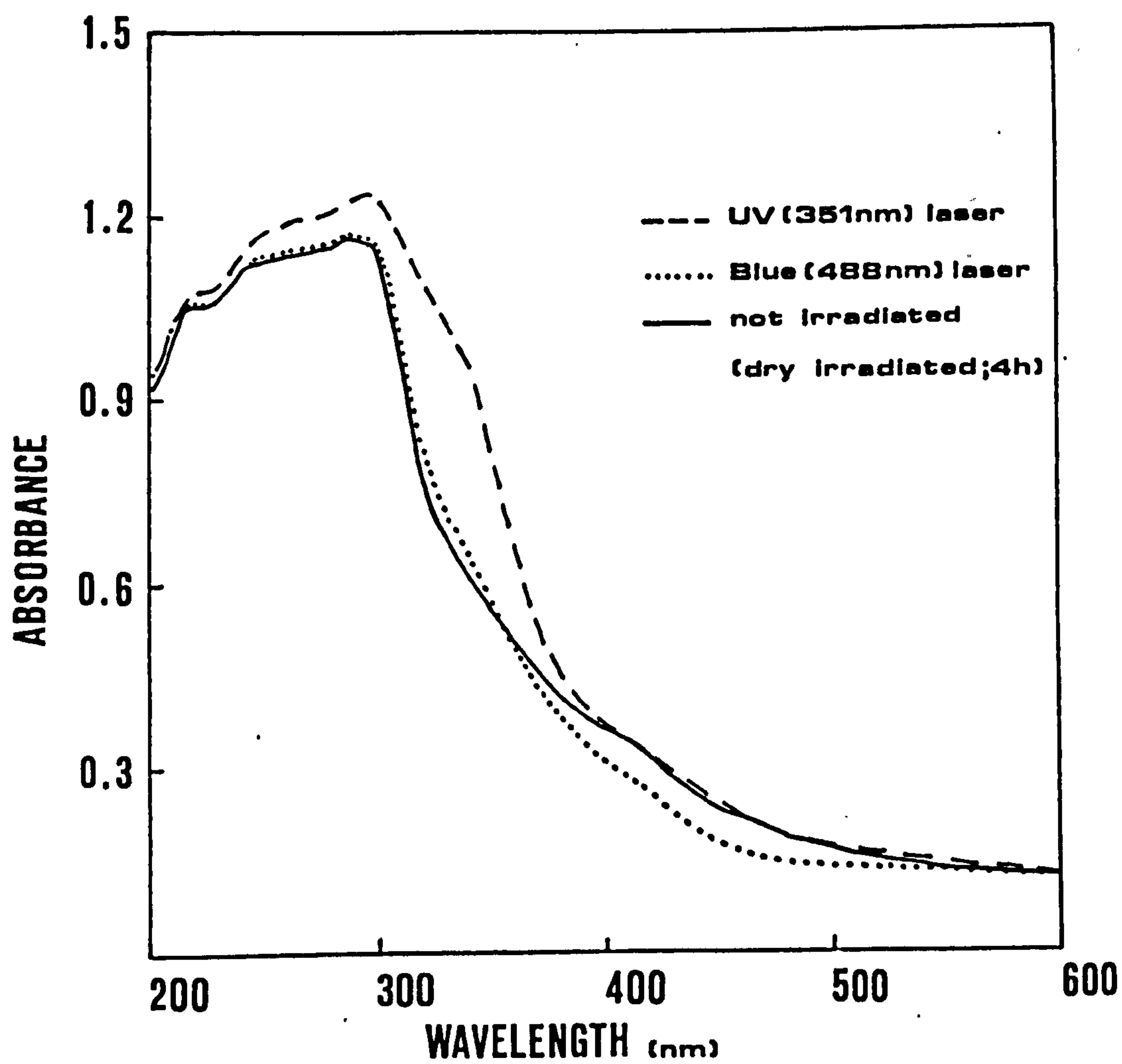


Figure 6.5 Diffuse reflectance spectra of wool fabric irradiated (dry) for 4 hours with various laser treatments.

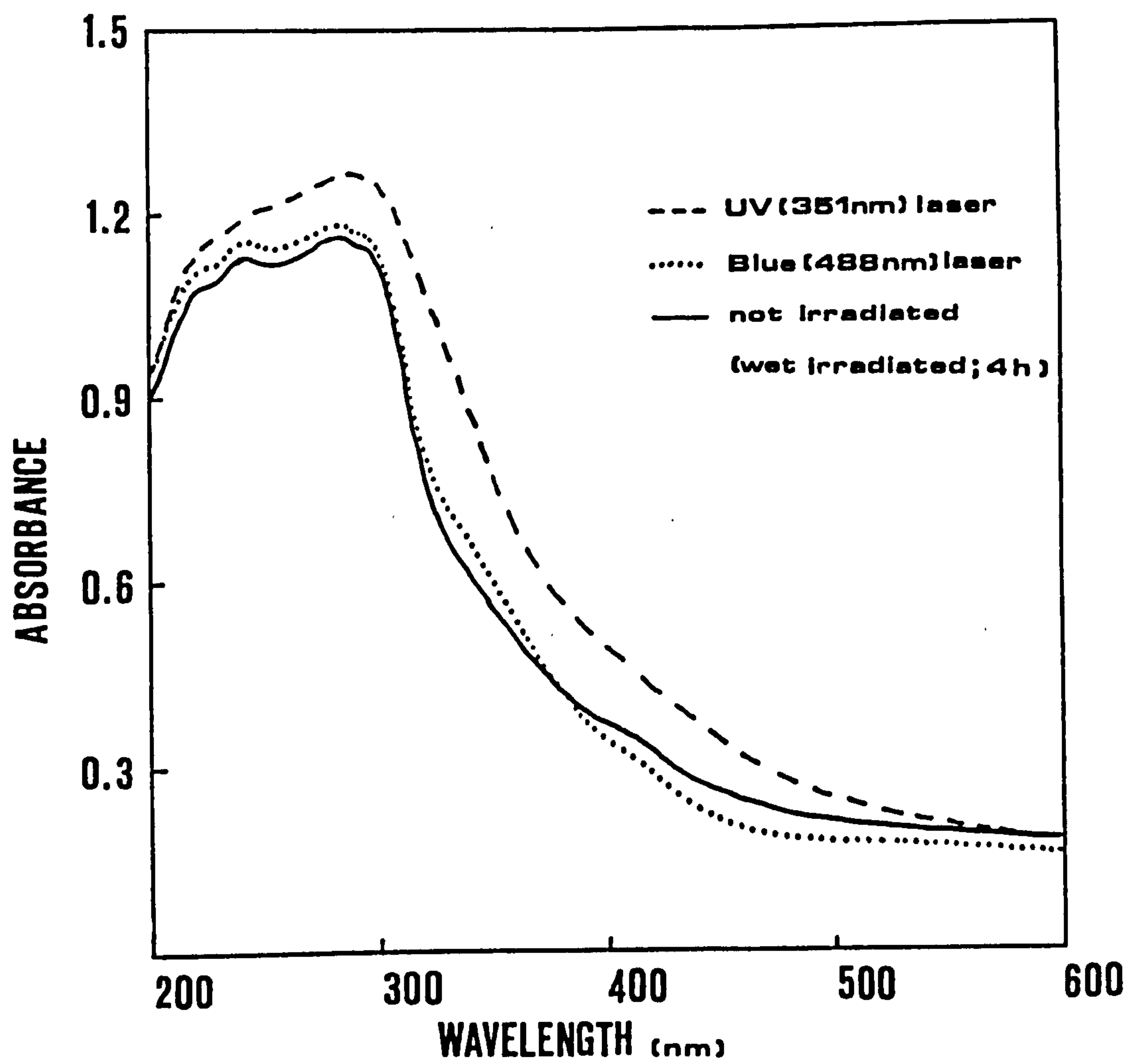


Figure 6.6 Diffuse reflectance spectra of wool fabric irradiated (wet) for 4 hours with various laser treatments.

Laser Line ^a	Time (hour)	State of Fabric	Fluorimeter Intensity ^{b,c}	YI ^d	Fluorescence Microscope Intensity	
					mean ^{b,c}	s.d. ^f
Blank	-	-	100.0	23.87	100.00	122.03
UV	1	dry	52.4	-	80.21	53.26
UV	4	dry	55.4	21.12	77.54	51.78
UV	1	wet	109.0	-	137.79	102.24
UV	4	wet	98.1	39.32	129.81	60.19
Blue	1	dry	101.3	-	51.85	37.06
Blue	4	dry	92.6	14.30	44.61	19.07
Blue	1	wet	100.9	-	36.77	13.78
Blue	4	wet	103.5	16.98	39.37	21.24

^a UV is 351 nm, 1.3-1.5 W; Blue is 488 nm, 7-8 W

^b arbitrary units ^c n=1 ^d Yellowness Index ^e n=50 ^f standard deviation

Table 6.3 Fluorescence intensities and Yellowness Index values of fabrics after various laser irradiation treatments.

An attempt was made to record the fluorescence intensity of the laser irradiated fabrics using a fluorescence spectrophotometer (fluorimeter) (Table 6.3). Although the area of the sample irradiated was larger than the area interrogated by the analysing light in the fluorimeter this was not particularly successful. This was due to the fact that the effect caused by the laser was not uniform but was more concentrated at the centre as revealed by the colour change (where there was one) of the fabric. For this reason subsequent measurements were made using micro-spectrofluorimetry. As fabrics are not very good samples for fluorescence microscopy (Section 6.3.5), portions of the fabrics, taken from the centre spot (5 mm diameter), were therefore used.

These exhibit the same properties as tops, namely that there is a large variation in the fluorescence intensities of fibres (Section 5.3.4). In an attempt to quantify any changes caused by the laser irradiations a large number of measurements (50) were made on the fibres removed from the centrally irradiated area. Not surprisingly the standard deviations are large. Using statistical analysis (t-test) to compare the blank with the 4 hours irradiated samples revealed no significant difference

($P=0.05$) for the UV irradiations but showed a significant difference ($P=0.05$ and $P=0.01$) for the blue light irradiations. The reduction in fluorescence intensity upon laser blue light irradiation agrees with the preliminary result using a 50 mW, 442 nm laser, but disagrees with the effect of Northlight irradiation (Section 6.3.3). The lack of a significant difference upon UV irradiation does not prove that a change has not taken place but rather that a change of any significance had not been observed. This result is not surprising when one considers the large standard deviations encountered.

An attempt was made to repeat the 15W argon ion laser investigations using 'clean' and 'dirty' staples. However, this proved fruitless since, even with low power blue light irradiation (1.2 W instead of 7 W; beam diameter 1 cm, sample dry), the 'dirty' wool staples easily burnt.

It is interesting to compare the results using laser irradiation with those obtained previously using UV lamps (Section 6.3.2) and Northlight lamps (Section 6.3.3). For the UV irradiation both laser and UV lamps produced similar results, leading to extensive yellowing and an increase in fluorescence (where a difference could be distinguished). For the blue light irradiation the results partly conflict - while both sources led to photobleaching, laser irradiation caused a decrease in fluorescence intensity while with Northlight lamps no change was observed.

A possible cause for the difference may be the respective energies of the light sources used although on inspection this does not seem to be the case. Using approximate calculations (and assuming 100% light output for lasers and 50% for Northlight lamps, as well as assuming that fabric is only one fibre thick which it is definitely not) the helium cadmium laser (50 mW, 390 min, 0.5 cm diameter) gives an energy density of 65 mW cm^{-2} and supplies 1.5 kJ cm^{-2} in the time used; the argon ion laser (15 W, 1-4 hour, 2 cm diameter) gives an energy density of 2.5 W cm^{-2} and supplies $9\text{-}36 \text{ kJ cm}^{-2}$ in the time used, and the Northlight lamps (3 x 20 W, irradiating an area $21 \times 57 \text{ cm}$ for up to 800 hours) gives an energy density of 25 mW cm^{-2} and supplies $0\text{-}70 \text{ kJ cm}^{-2}$. Hence, the energy supplied by the lasers is the same or less than that supplied by the lamps and yet they produce a decrease in fluorescence intensity while the Northlight lamps do not! An

additional possibility is that the high intensity of the laser light causes effects such as two photon processes which cannot happen when using low intensity lamps.

An alternative possible cause for the difference may be the heating of the wool by the light sources. Bahnert and Schollmeyer [12] found that UV laser irradiation of wool (193 nm, 300 pulses at 68 mJ cm^{-2} giving 20.4 J cm^{-2}) led to the formation of a substructure on the fibre surface and attributed this to superheating ($> 1000\text{K}$) and surface melting. It is known that dry heat ($\geq 120^\circ\text{C}$) causes damage to wool [13]. However, heating wool at 115°C has shown to lead to an increase in the natural fluorescence of wool [6], due to the destruction of tryptophan [8]. Admittedly, 115°C is somewhat different from superheating but the opposite effect to what would be expected on a heat basis is observed (Table 6.3).

Consequently, how laser light interacts with wool on a fibre/molecular basis is unclear and further work could usefully be directed towards investigating this as well as the effect of multiple irradiation using UV and then blue light (and vice versa). However, the effect is more obvious: 351 nm UV irradiation leads to yellowing, manifested by an increase in both visible and UV absorbing chromophores with no significant difference in the level of natural fluorescence; 488 nm blue light irradiation leads to photobleaching, manifested by a destruction of visible absorbing chromophores and a modest increase in UV absorbing chromophores, with a significant difference in the level of natural fluorescence (a decrease).

6.3.5 Other Investigations

A comparison of the fluorescence spectra and intensities of wool fabric subjected to various treatments was undertaken. Those used consisted of untreated fabric, oxidatively bleached fabric, laser blue light irradiated fabric (50 mW, 442 nm helium cadmium laser, 390 min), and UV light irradiated fabric (irradiated *in situ* on the microscope) (Figure 6.7).

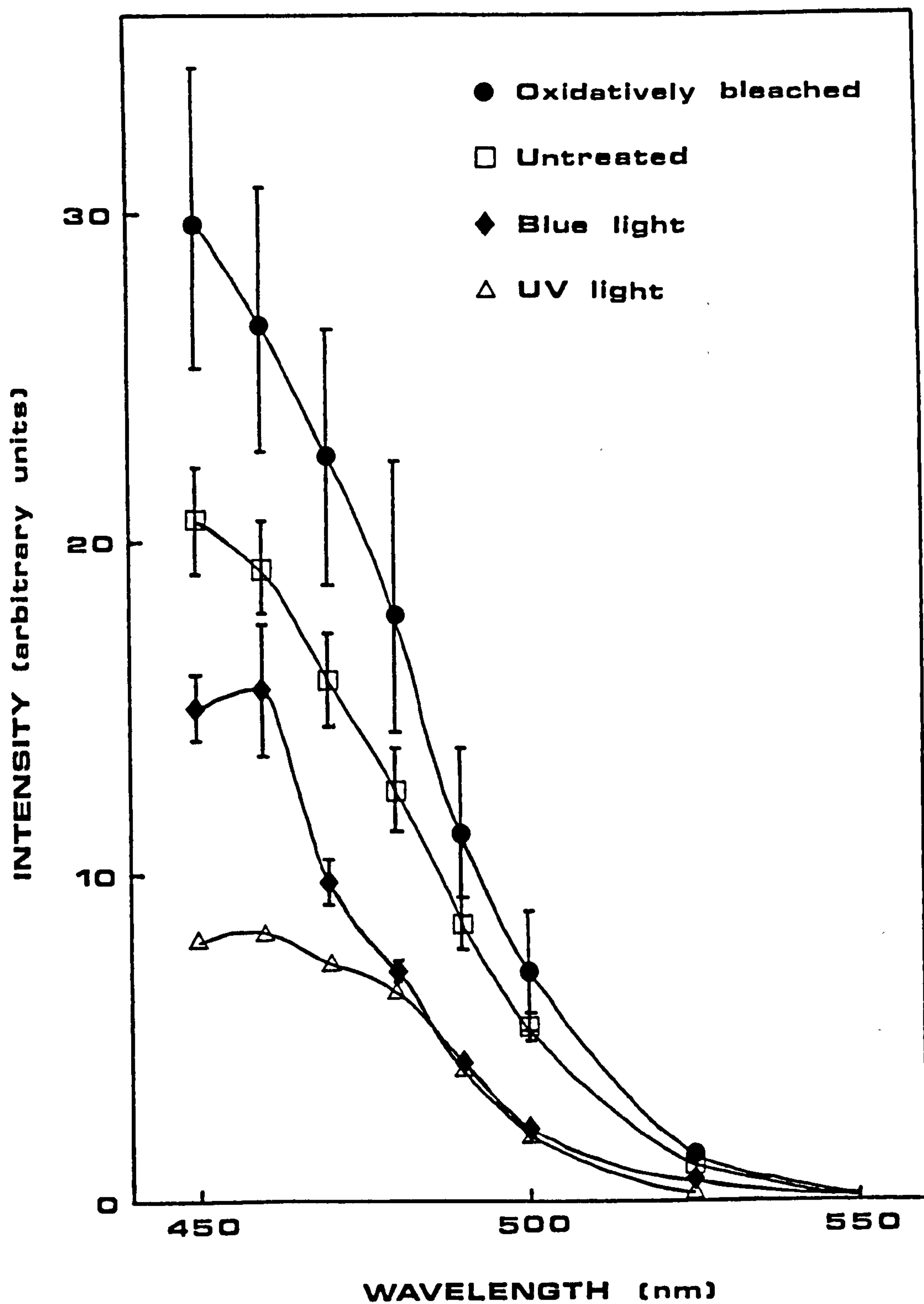


Figure 6.7 Fluorescence spectra of wool fabric which had been subjected to various treatments (UV excitation, mean \pm standard error of the mean).

The spectra of all the treatments are very similar which agrees with the comment in Section 2.5.2.1 that the fluorescence spectra of wool is almost independent of the treatment it receives. The fluorescence intensity of the wool after the different treatments is a different matter. Oxidative bleaching caused an increase in intensity which agrees with the earlier finding (Section 5.3.4), as well as other work (Section 2.5.2.2) and this increase can be attributed to conversion of quenching cystine to non-quenching cysteic acid. Laser blue light irradiation caused a decrease in fluorescence intensity. This has just been discussed in detail in Section 6.3.4. Irradiation of fabric (embedded in immersion oil (a hydrocarbon oil [14])) on the microscope stage with UV excitation source led to a decrease in fluorescence intensity. This is surprising, as previous results (Section 6.3.2) have shown an increase in fluorescence intensity upon UV irradiation. Consequently, a study of the role of the embedding medium was undertaken.

Wool fabric immersed in immersion oil or water was irradiated (non *in situ*) using Blacklight lamps producing UV light (Table 6.4). Compared with both the water sample and a blank, the immersion oil caused a significant difference in fluorescence (t-test, $P=0.05$ and $P=0.01$). The irradiation in water caused the Yellowness Index (YI) of the fabric to increase from 20.20 to 30.85 and the fluorescence emission maximum to shift to 460 nm. However, the YI of the fabric irradiated in immersion oil increased by only a small amount to 22.50 and the emission maximum only shifted to 445 nm. Similar fluorescence results were obtained when glycerol was used instead of immersion oil (not shown). Then the fabric irradiated in glycerol increased in YI from 18.07 to 19.39 and smelt 'sulphury' while the YI of the water irradiated fabric increased to 34.07.

Irradiation Condition	Yellowness Index	Approximate Peak Emission ^a (nm)	Fluorescence mean ^b	Intensity s.d. ^c
Blank	20.20	440	100.0	13.6
Immersion oil	22.50	445	67.3	14.6
Water	30.85	460	115.4	12.9

^a excitation 360 nm ^b arbitrary units ^c standard deviation

Table 6.4 Yellowness Index values, fluorescence intensities and peak emissions of wool fabrics irradiated with UV light in different embedding mediums.

Bailey and Launer [15], using a Westinghouse 'Blacklight' fluorescent lamp, found that if the strongly yellowing spectral impurity of 313 nm was filtered out the main radiation near 365 nm caused photobleaching. As 365 nm light is generally regarded as causing yellowing (Section 2.4.3.2; Figure 2.7) and was used as such in UV irradiations, notably in Section 6.3.2 as well as here, this was a cause for concern. At the same time as the immersion oil was investigated non *in situ* the effect of the 313 and 365 nm wavelength radiations was investigated. The experiment was done in duplicate - once with laminated glass to filter out the 313 nm radiation, and once without any filter. Table 6.5 shows the results obtained (the ones without any filter are the ones reported in Table 6.4).

Using a filter, the irradiation in water caused the YI of the fabric to increase from 20.20 to 35.44 and the fluorescence emission maximum to shift to 460 nm while the YI of the fabric irradiated in immersion oil increased by only a small amount to 22.54 and the emission maximum only shifted to 445 nm, i.e. the same results as obtained without using a filter. For the filtered fabrics, the immersion oil caused a significant difference in fluorescence (t-test, $P=0.05$ and $P=0.01$) when compared with both the water sample and the blank, again the same as for the unfiltered fabrics. Additionally, there was found to be no significant difference (t-test, $P=0.05$) between the two immersion oil samples, or between the two water samples. Therefore, 365 nm radiation, and consequently Blacklight/UV lamps as well as microscope mercury lamps, do cause photoyellowing of wool as expected, and the claims of Bailey and Launer are disproved.

Irradiation Condition	Yellowness Index	Approximate Peak Emission ^a (nm)	Fluorescence mean ^b	Intensity s.d. ^c
Blank	20.20	440	100.0	13.6
Immersion oil	22.50	445	67.3	14.6
Immersion oil + filter	22.54	445	75.7	12.9
Water	30.85	460	115.4	12.9
Water + filter	35.44	460	113.1	12.8

^a excitation 360 nm ^b arbitrary units ^c standard deviation

Table 6.5 Yellowness Index values, fluorescence intensities and peak emissions of wool fabrics irradiated with UV light in different embedding mediums with or without a laminated glass filter.

During the comparison measurements on the microscope (Figure 6.7) it was found that fabrics are not good materials for microspectrofluorimetry. As fabrics are made of threads a relatively high depth of field was required to view them, which means a low power objective (5x) had to be used. Hence, the advantages of microscope analysis are largely lost and so the use of a fluorescence spectrophotometer is more suitable. Consequently, (whole) fabric fluorescence analysis was then restricted to the fluorescence spectrophotometer.

The effect of a number of treatments on wool have been compared. Oxidative bleaching caused an increase in the natural fluorescence, laser blue light caused a decrease and *in situ* UV light irradiation also caused a decrease. This last, unexpected, result was established as arising from the influence of the embedding medium used. The use of immersion oil or glycerol caused the fluorescence intensity of the wool to decrease. 365 nm radiation was confirmed as causing photoyellowing, while it was found that fabrics are not good samples for inspection using microspectrofluorimetry. This reiterates the need for care to be taken in sample preparation for microspectrofluorimetry.

6.4 CONCLUSIONS

Irradiation with sunlight causes the difference in root-tip visible fluorescence to disappear with the median level of fluorescence increasing. The UV and blue light components of the spectrum have different effects on the natural fluorescence of wool. UV irradiation of wool using UV fluorescent lamps caused a rapid increase in the fluorescence intensity (arising from fluorescent tryptophan degradation products [5,6]) which was accompanied by a definite protective effect being exhibited by the wool yolk. This was complemented by 351 nm laser irradiation where extensive yellowing was produced, as manifested by an increase in both visible and UV absorbing chromophores, but with no significant difference in the level of natural fluorescence being observed (due to the large standard deviations encountered). Blue light irradiation with Northlight fluorescent lamps resulted in photobleaching yet with no change being observed in the fluorescence intensity and the wool yolk having no apparent protective effect. Although with 488 nm laser irradiation visible absorbing chromophores were destroyed, along with a modest increase in UV absorbing chromophores, the natural fluorescence decreased. The reasons for the differences between the two types of blue light sources is not clear, although it is clear that the enhancement of white appearance in photobleaching is not due to the production of UV absorbing species which exhibit blue fluorescence but to the destruction of yellow coloured residues. The level of natural fluorescence of wool decreases when wool, embedded in immersion oil or glycerol, is irradiated with UV light.

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CHAPTER 7

NATURAL FLUORESCENCE OF WOOL

-EFFECT OF REDUCING AGENTS

7.1 INTRODUCTION

Many factors sensitise wool to photoyellowing, including being (oxidatively) chemically bleached, and treatment with Fluorescent Whitening Agents (Section 2.4.3.2 and Figure 2.11). Many investigators, appreciating that the photoyellowing of wool is an oxidation reaction, have attempted to minimise the yellowing of wool and fluorescent whitened wool with reducing agents (Section 2.4.5.2 Part 7). Several classes of reducing agents have been examined, which under certain conditions minimise the photoyellowing of wool. The protective effect is most evident if the samples are kept wet during exposure and is especially marked if the wool is actually irradiated in a solution of the reducing agent. Both UV and visible light have been used.

Little work has been carried out on the effect of reducing agents on the natural fluorescence of wool (Section 2.5.2.2). Jones [1] found that UV irradiation of wool in Blankit D solution caused the wool to lose approximately half of its fluorescence, while Leaver [2] observed a marked decrease in the fluorescence of untreated (one half) and oxidatively bleached (one third) wools upon treatment with thiourea/formaldehyde. In the previous chapter, Chapter 6, blue light photobleaching of untreated wool was investigated. It was found that the enhancement of white appearance in photobleaching was not due to the production of UV absorbing species exhibiting blue fluorescence but to the destruction of yellow coloured residues.

This chapter describes a series of experiments which have been carried out in order to obtain a better understanding of the effect of reducing agents on wool (especially on its natural fluorescence). The effect of using reducing agents with UV irradiation, and with visible irradiation, has been investigated as well as their effect on their own (in order to establish the degree of change due to chemical action alone).

7.2 EXPERIMENTAL

A detailed explanation of the main materials and techniques used can be found in Chapter 4.

Unbleached fabric and fabric which had been oxidatively bleached using Bleaching Treatment 1 were used. The reducing agents used were Rongalit C (BASF), Blankit D (BASF), sodium hypophosphite ($\text{NaH}_2\text{PO}_2 \cdot x\text{H}_2\text{O}$) (Aldrich) and thiourea dioxide ($\text{H}_2\text{NC}(=\text{NH})\text{SO}_2\text{H}$, also known as formamidine sulphinic acid and aminoiminomethane sulphinic acid) (Aldrich) as well as distilled water. Blankit D consists of 80% zinc formaldehyde sulfoxylate ($\text{HOCH}_2\text{S}(=\text{O})\text{OZnOS}(=\text{O})\text{CH}_2\text{OH}$) and, 20% stabilisers and complexing agents [1]. Rongalit C is similar except that it contains the sodium salt ($\text{Na}^{\oplus}\text{OS}(=\text{O})\text{CH}_2\text{OH}$).

For treatment with reducing agent two pieces of fabric (one unbleached, one oxidatively bleached, each approximately 6 x 10cm) were placed on a glass plate, 125 x 100mm, (in a white china dish) after being dipped in aqueous Lissapol N and rinsed. The ends of the fabric were covered with aluminium foil to keep the fabrics in place. During treatment the fabrics were immersed under 2cm of 2% (w/v) solution of reducing agent or distilled water alone. After treatment they were rinsed in water and then air dried in the dark at room temperature.

Three processes were used for treating the fabrics in the solutions. The first involved irradiating for 24 hours using Irradiation Method 1 equipped with Blacklight lamps (Philips, 3 x 18W, 600mm). The second process involved irradiating for 24 hours using Irradiation Method 1 equipped with Northlight 55 lamps (Philips, 3 x 20W, 600mm) and having laminated glass (6mm) as a UV filter (but this did not prevent air movement around the containers). The third process involved leaving for 24 hours in the dark in a cupboard.

The fluorescence intensities of the wool fabrics were obtained using a MPF-4 fluorescence spectrophotometer (Section 4.4.1). The samples were excited at 360 nm and the fluorescence emission measured between 370 and 610 nm. For each of the pieces of fabrics three portions were measured. Yellowness Index and CIE tristimulus values of the fabrics were determined and diffuse reflectance spectra obtained.

7.3 RESULTS AND DISCUSSION

7.3.1 Effect of UV and Reducing Agents

Fabrics (unbleached and oxidatively bleached) were immersed in 2% solutions of various reducing agents and exposed to UV irradiation for 24 hours. Diffuse reflectance spectra obtained from the samples are shown in Figure 7.1 (unbleached fabric) and Figure 7.2 (oxidatively bleached fabric).

For unbleached fabric, water, Rongalit C and sodium hypophosphite treated fabrics exhibit a small increase in visible absorbing chromophores (hence becoming yellower) and a medium increase in UV absorbing chromophores, while Blankit D and thiourea dioxide treated fabrics exhibit a large decrease in both visible (hence becoming whiter) and UV absorbing chromophores.

For oxidatively bleached fabric (Figure 7.2) similar trends were observed as for the unbleached fabric, although the magnitudes of all the changes were not the same. Rongalit C treated fabric exhibits a small increase in visible absorbing chromophores (hence becoming yellower) and a medium increase in UV absorbing chromophores; water and sodium hypophosphite treated fabrics exhibit a medium increase in visible absorbing chromophores (hence becoming yellower) and a large increase in UV absorbing chromophores, while Blankit D and thiourea dioxide treated fabrics exhibit a medium decrease in visible absorbing chromophores (hence becoming whiter) and a large decrease in UV absorbing chromophores.

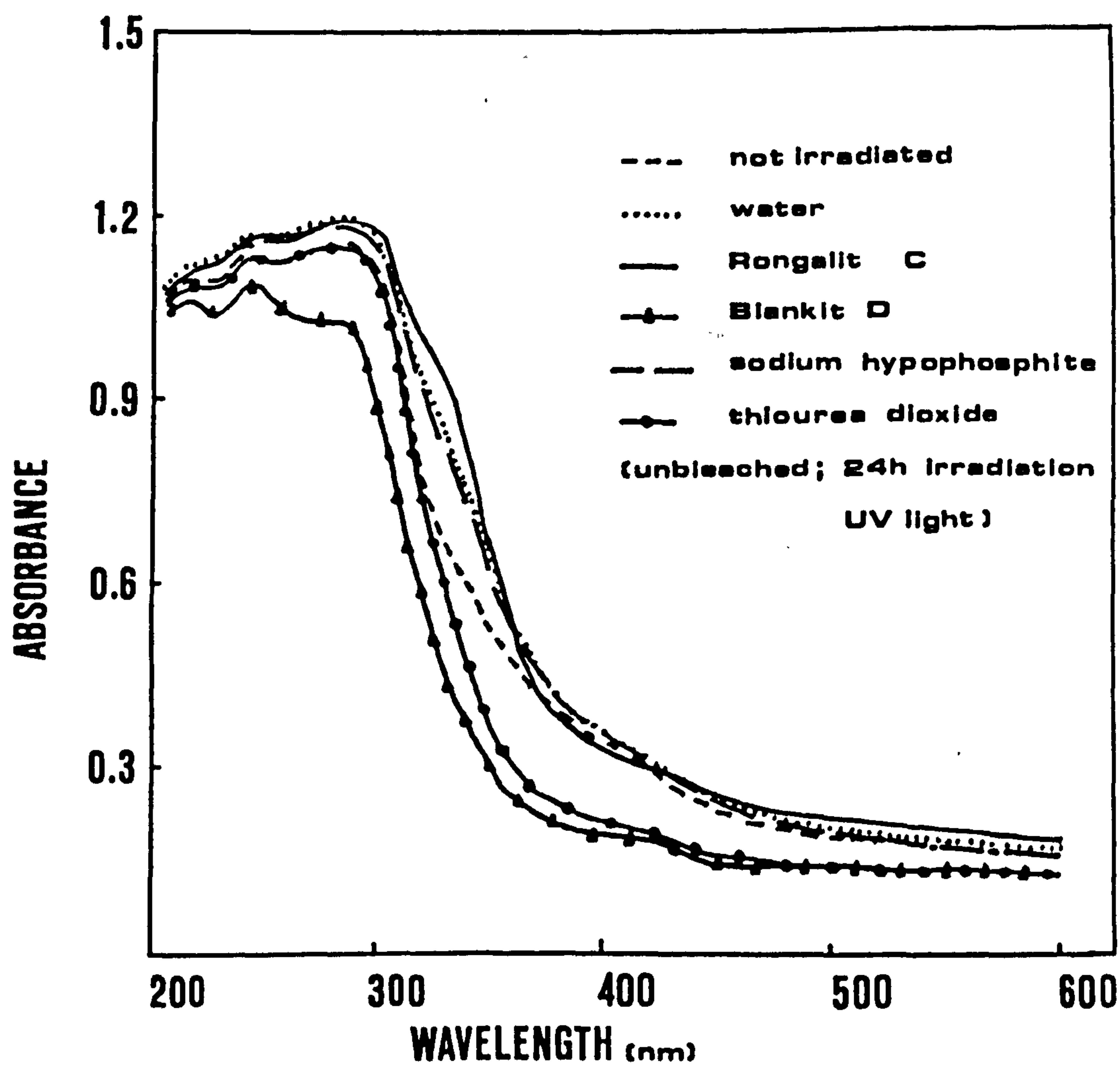


Figure 7.1 Diffuse reflectance spectra of unbleached fabric after UV irradiation for 24 hours in 2% solutions of various reducing agents.

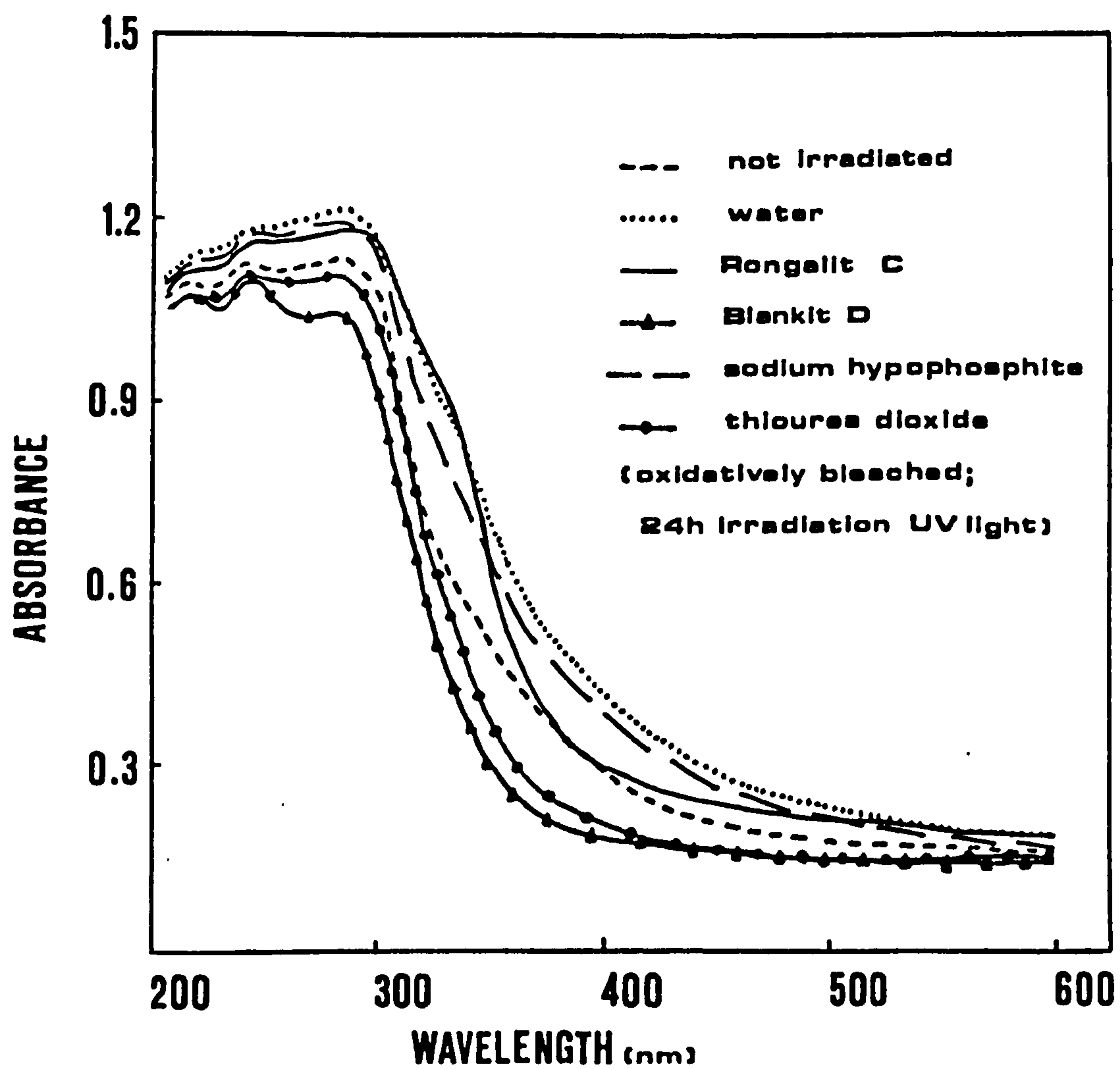


Figure 7.2 Diffuse reflectance spectra of oxidatively bleached fabric after UV irradiation for 24 hours in 2% solutions of various reducing agents.

Solution	Fluorescence		Colour Measurements		
	mean ^a	standard deviation	Yellowness Index	Y	Y - Z
Not irradiated	100.0	9.5	22.01	72.33	9.15
Water	95.9	11.9	24.05	69.77	9.98
Rongalit C	45.0	1.1	22.03	67.05	7.93
Blankit D	58.6	4.0	8.61	79.12	0.68
Sodium hypophosphite	108.3	6.2	24.31	69.73	10.01
Thiourea dioxide	93.1	10.3	11.45	77.46	2.79

^a arbitrary units

Table 7.1 Relative fluorescence intensities (360 nm excitation) and colour measurements of unbleached fabric after UV irradiation for 24 hours in 2% solutions of various reducing agents.

The colour of the treated fabrics was quantified using Yellowness Index (YI) values and CIE tristimulus values (Tables 7.1 and 7.2). For unbleached fabric (Table 7.1), water, Rongalit C and sodium hypophosphite treated fabrics manifest a small increase in YI (0.02 to 2.30), while Blankit D and thiourea dioxide treated fabrics manifest a large decrease in YI (10.56 and 13.40). The Y-Z values (providing an indication of yellowness) however provided slightly different results. Water and sodium hypophosphite treated fabrics showed an increase in Y-Z value, and, Blankit D and thiourea dioxide treated fabrics showed a decrease in Y-Z value, which agrees with the YI results. However, the Rongalit C treated fabric showed a decrease in Y-Z value i.e. the fabric had become less yellow! The Y values (providing an indication of brightness) have the reverse pattern to the YI values i.e. the more yellow the fabric the less bright it is. Water, Rongalit C and sodium hypophosphite treated fabrics manifest a decrease in Y value, while Blankit D and thiourea dioxide treated fabrics manifest an increase in Y value.

Solution	Fluorescence		Colour Measurements		
	mean ^a	standard deviation	Yellowness Index	Y	Y - Z
Not irradiated	180.5	13.3	15.63	74.35	5.08
Water	122.8	4.5	25.98	67.94	10.43
Rongalit C	61.4	5.9	18.86	68.36	6.13
Blankit D	61.6	7.6	6.29	80.15	-1.03
Sodium hypophosphite	150.7	6.6	27.65	70.59	11.80
Thiourea dioxide	96.6	4.1	9.31	77.55	1.13

^a arbitrary units

Table 7.2 Relative fluorescence intensities (360 nm excitation) and colour measurements of oxidatively bleached fabric after UV irradiation for 24 hours in 2% solutions of various reducing agents.

For oxidatively bleach fabric (Table 7.2) similar trends are observed as for the unbleached fabric except that the anomaly with the Rongalit C treated fabric between the YI and Y-Z value is absent. It should be remembered that YI values, CIE tristimulus values and diffuse reflectance spectra are all attempts to measure colour (see Section 2.4.3) and do not necessarily give the same result. However, there is good general agreement between the different colour measurements here: for both unbleached and oxidatively bleached fabrics, water, Rongalit C and sodium hypophosphite treatments show an increase in yellowness together with a decrease in brightness, while Blankit D and thiourea dioxide treatments show a decrease in yellowness together with an increase in brightness. The anomaly with the Rongalit C treatment of unbleached fabric may be explained by the observation in the diffuse reflectance spectra (Figure 7.1) that the absorption of the treated fabric dips briefly around 400 nm, with the difference in the YI and Y-Z values arising from the different ways they are calculated.

The fluorescence intensities of the fabrics were also determined (Tables 7.1 and 7.2). For unbleached fabric (Table 7.1), water, sodium hypophosphite and thiourea dioxide treated fabrics manifest no notable change in fluorescence intensity while Rongalit C and Blankit D treated fabrics manifest a large decrease in fluorescence intensity. For oxidatively bleached fabric (Table 7.2), sodium hypophosphite treated fabric manifests a small change in fluorescence intensity, water and thiourea dioxide treated fabrics manifest a large decrease in fluorescence intensity, while Rongalit C and Blankit D treated fabrics manifest a very large decrease in fluorescence intensity. There are two observable trends. The first being that all treatments cause the fluorescence intensity of oxidatively bleached fabric to decrease. This suggests that the increased level of fluorescence due to oxidative bleaching is unstable. The second trend being that Rongalit C and Blankit D treated fabrics, unbleached and oxidatively bleached, display a large decrease in fluorescence intensity.

These results generally agree with the few previous studies. For unbleached fabric photoyellowing has been observed upon treatment with water [3,4], while photobleaching has been observed upon treatment with Blankit D [1,5], thiourea dioxide [3,4], and, disagreeing with the present results, Rongalit C [1,4] and sodium hypophosphite [3]. Concerning the fluorescence, only Blankit D treated fabric has been investigated [1], and this was found to exhibit a decrease in the level of intensity of fluorescence of about half (the effect of UV light on the fluorescence intensity of wool has been considered in detail in Section 2.5.2.2 and Chapter 6). For oxidatively bleached fabric, photoyellowing has been observed upon treatment with water [4], and, disagreeing with the present results, Blankit D [1], while photobleaching has been observed upon treatment with thiourea dioxide [4]. The fluorescence has not been investigated, except that it is known that oxidative bleaching causes an increase in the natural fluorescence of wool (Section 2.5.2.2).

It is difficult to correlate the colour and fluorescence intensities of the treated fabrics, although it is clear that the treatments do not cause an increase in the level of fluorescence, and that any increase in whiteness/decrease in yellowness is not due to the production of new fluorescent species.

These results are considered further in Section 7.3.4.

7.3.2 Effect of Blue Light and Reducing Agents

Fabrics (unbleached and oxidatively bleached) were immersed in 2% solutions of various reducing agents and exposed to blue light irradiation for 24 hours. Diffuse reflectance spectra obtained from the samples are shown in Figure 7.3 (unbleached fabric) and Figure 7.4 (oxidatively bleached fabric).

For unbleached fabric, water, Rongalit C and sodium hypophosphite treated fabrics exhibit a small increase in visible absorbing chromophores above approximately 480 nm, a small decrease or no change in absorbing chromophores between approximately 380 and 480 nm, and a small increase in UV absorbing chromophores below approximately 380 nm, while Blankit D and thiourea dioxide treated fabrics exhibit a medium decrease in visible absorbing chromophores (hence becoming whiter) and a small decrease in UV absorbing chromophores.

For oxidatively bleached fabric (Figure 7.4) similar trends were observed as for the unbleached fabric. Water, Rongalit C and sodium hypophosphite treated fabrics exhibit a small increase in visible absorbing chromophores above approximately 490 nm, a small decrease or no change in absorbing chromophores between 390 and 490 nm, and a small increase in UV absorbing chromophores below approximately 390 nm, while Blankit D and thiourea dioxide treated fabrics exhibit a medium decrease in visible absorbing chromophores (hence becoming whiter) and a medium decrease in UV absorbing chromophores.

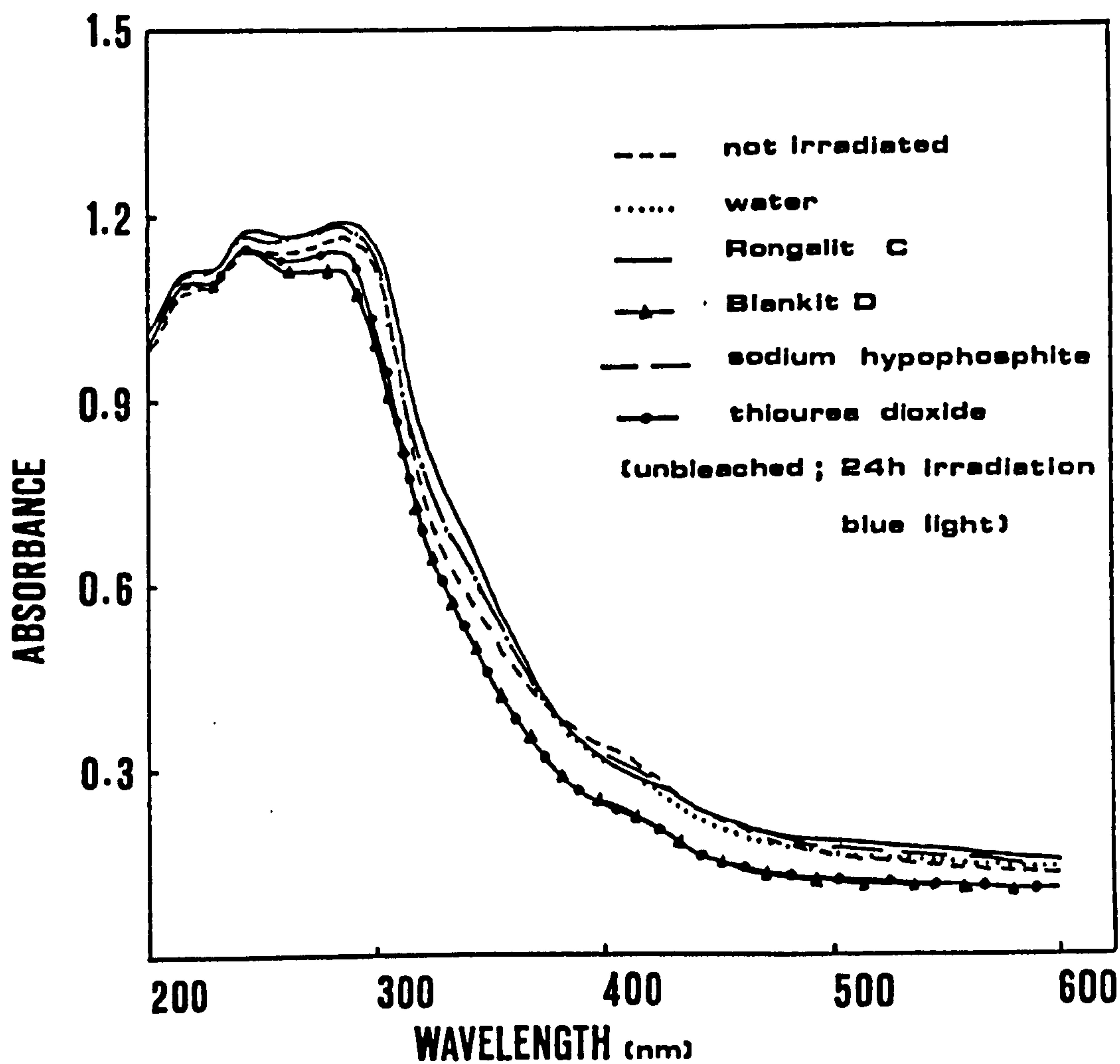


Figure 7.3 Diffuse reflectance spectra of unbleached fabric after blue light irradiation for 24 hours in 2% solutions of various reducing agents.

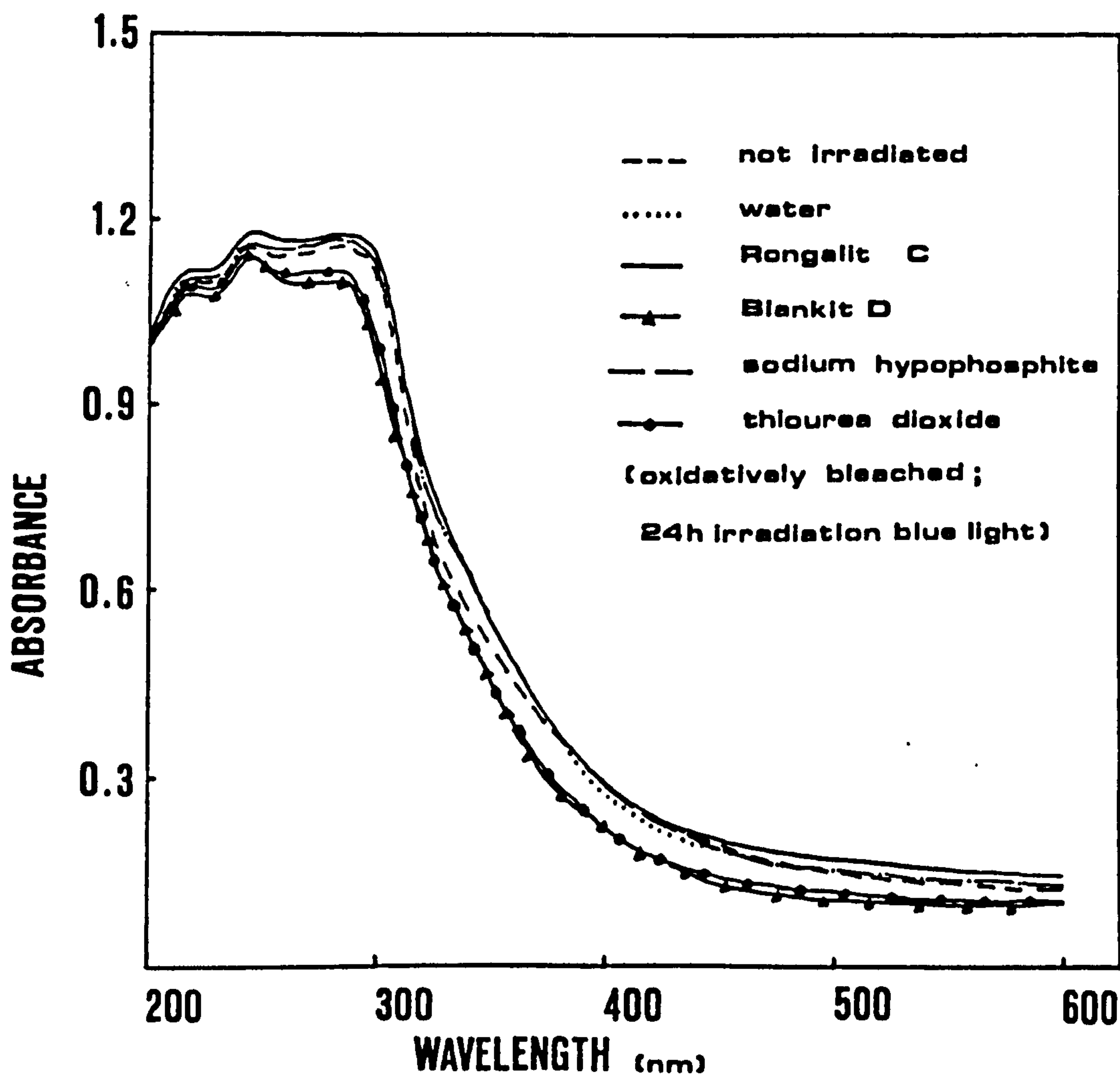


Figure 7.4 Diffuse reflectance spectra of oxidatively bleached fabric after blue light irradiation for 24 hours in 2% solutions of various reducing agents.

Solution	Fluorescence		Colour Measurements		
	mean ^a	standard deviation	Yellowness Index	Y	Y - Z
Not irradiated	100.0	7.4	20.84	72.08	8.46
Water	119.0	3.8	16.54	71.17	5.57
Rongalit C	112.6	6.3	17.65	68.82	5.63
Blankit D	107.7	6.6	11.72	76.22	2.94
Sodium hypophosphite	129.3	4.6	17.29	71.32	5.99
Thiourea dioxide	161.0	10.0	12.37	75.35	3.33

^a arbitrary units

Table 7.3 Relative fluorescence intensities (360 nm excitation) and colour measurements of unbleached fabric after blue light irradiation for 24 hours in 2% solutions of various reducing agents.

The colour of the treated fabrics was quantified using YI and CIE tristimulus values (Tables 7.3 and 7.4). For unbleached fabric (Table 7.3), all treatments resulted in the fabrics manifesting a decrease in YI (3.19 to 9.12), with Rongalit C and sodium hypophosphite causing a smaller decrease than water (3.19 and 3.55 compared with 4.30), and Blankit D and thiourea dioxide causing a larger decrease than water (9.12 and 8.47 compared with 4.30). The Y-Z values (providing an indication of yellowness) show the same pattern. The Y values (providing an indication of brightness) provided slightly different results. Water, Rongalit C and sodium hypophosphite treated fabrics manifest a decrease in Y value, while Blankit D and thiourea dioxide treated fabrics manifest an increase in Y value.

Solution	Fluorescence		Colour Measurements		
	mean ^a	standard deviation	Yellowness Index	Y	Y - Z
Not irradiated	172.5	1.2	16.17	73.41	5.46
Water	170.1	5.9	12.36	72.55	2.71
Rongalit C	153.2	11.6	14.46	69.80	3.72
Blankit D	146.4	3.4	8.36	77.73	0.63
Sodium hypophosphite	176.1	6.6	15.07	72.28	4.43
Thiourea dioxide	185.3	15.6	8.43	77.23	0.52

^a arbitrary units

Table 7.4 Relative fluorescence intensities (360 nm excitation) and colour measurements of oxidatively bleached fabric after blue light irradiation for 24 hours in 2% solutions of various reducing agents.

For oxidatively bleached fabric (Table 7.4) the same trends are observed as for the unbleached fabric. There is good general agreement between the different colour measurements here: for both unbleached and oxidatively bleached fabrics, all treatments show a decrease in yellowness i.e. they became whiter, with Rongalit C and sodium hypophosphite treatments showing a smaller decrease in yellowness and a lower (or similar) level of brightness than water alone, while Blankit D and thiourea dioxide treatments show a larger decrease in yellowness and a higher level of brightness than water alone.

The fluorescence intensities of the fabrics were also determined (Table 7.3 and 7.4). For unbleached fabric (Table 7.3), Rongalit C and Blankit D treated fabrics manifest no notable change in fluorescence intensity; water and sodium hypophosphite treated fabrics manifest a small increase in fluorescence intensity and thiourea dioxide treated fabrics manifest a large increase in fluorescence intensity. For oxidatively bleached fabric (Table 7.4), water, sodium hypophosphite and thiourea dioxide treated fabrics manifest no notable change in

fluorescence intensity, while Rongalit C and Blankit D treated fabrics manifest a small decrease in fluorescence intensity. Although, there is an anomaly with unbleached fabric treated with thiourea dioxide, there is a general trend that all the treatments cause little, if any, change in the fluorescence intensity of the fabrics.

These results agree with the few previous studies. Photobleaching has been observed for unbleached fabric upon treatment with Rongalit C [1], as well as for (presumably unbleached) fabric upon treatment with sodium hypophosphite [6] and thiourea dioxide [6], while water is known to promote photobleaching of unbleached wool [7,8]. Interestingly, photobleaching has also been observed upon dry irradiation of oxidatively bleached fabric, thiourea dioxide bleached fabric, sodium formaldehyde sulfoxylate bleached fabric and zinc formaldehyde sulfoxylate treated fabric, in each case to a greater extent than the control [8]. No work appears to have been reported concerning the effect of the treatments on the fluorescence (the effect of blue light on the fluorescence intensity of wool has been considered in detail in Section 2.5.2.2 and Chapter 6).

There is no correlation between the colour and fluorescence intensities of the treated fabrics, although this is hardly surprising given the finding of Chapter 6 (Section 6.4) that (non-laser) blue light irradiation results in 'photobleaching yet with no change being observed in the fluorescence intensity'. The treatments may simply be promoting (or hindering) the effect of the blue light irradiation.

These results are considered further in Section 7.3.4.

7.3.3 Effect of Reducing Agents Alone

Fabrics (unbleached and oxidatively bleached) were immersed in 2% solutions of various reducing agents and kept in the dark in a cupboard for 24 hours. Diffuse reflectance spectra obtained from the samples are shown in Figure 7.5 (unbleached fabric) and Figure 7.6 (oxidatively bleached fabric).

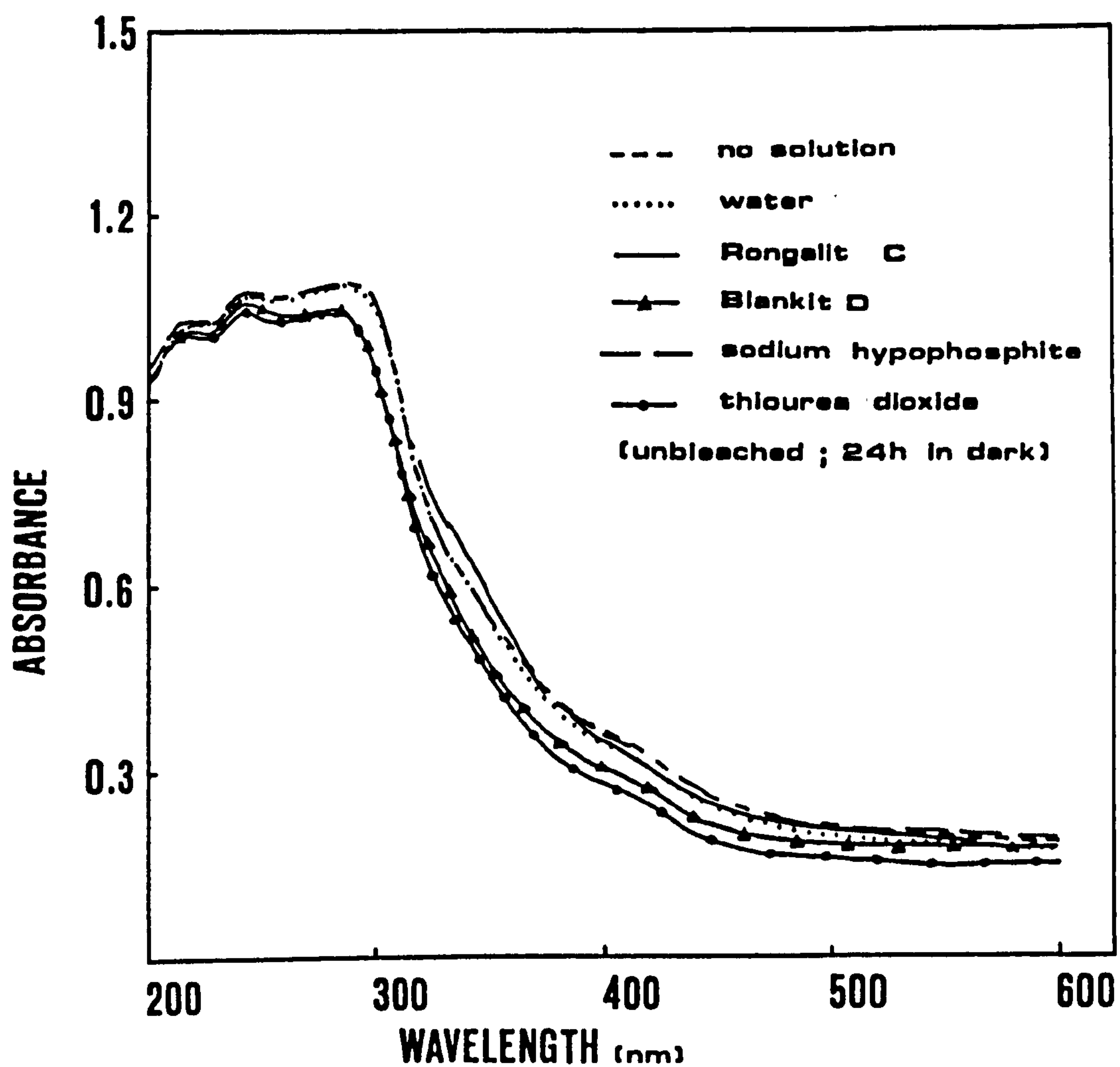


Figure 7.5 Diffuse reflectance spectra of unbleached fabric after being kept in the dark for 24 hours in 2% solutions of various reducing agents.

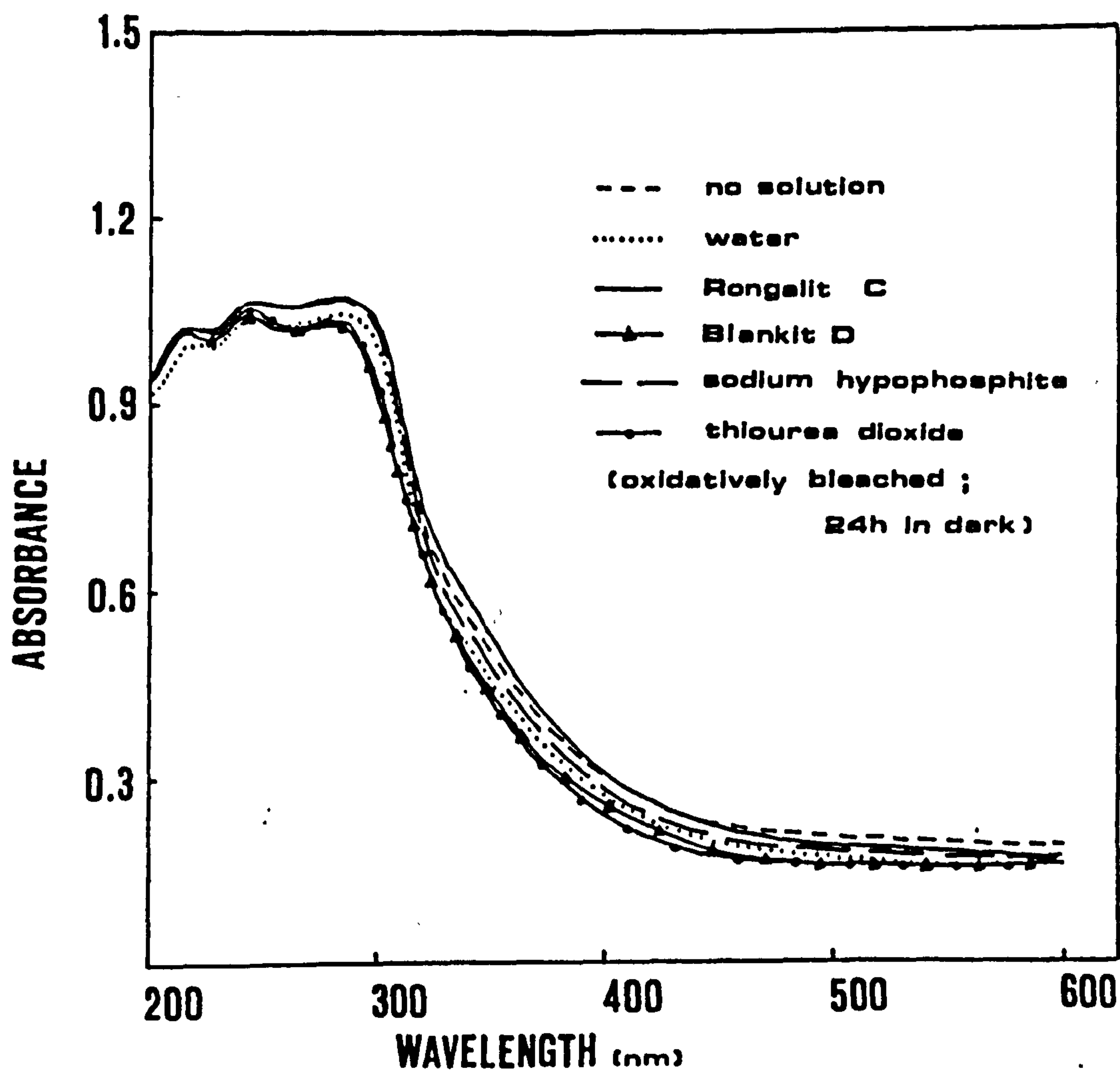


Figure 7.6 Diffuse reflectance spectra of oxidatively bleached fabric after being kept in the dark for 24 hours in 2% solutions of various reducing agents.

For unbleached fabric, sodium hypophosphite treated fabric exhibits a small increase in visible absorbing chromophores above approximately 520 nm, with almost no change below 520 nm; water treated fabric exhibits a small decrease in absorbing chromophores above approximately 350 nm (hence becoming whiter), with no change below 350 nm; Rongalit C treated fabric exhibits a small decrease in absorbing chromophores above approximately 370 nm (hence becoming whiter), with no change or a small increase below 370 nm, while Blankit D and thiourea dioxide treated fabrics exhibit a medium decrease in both visible absorbing chromophores (hence becoming whiter) and UV absorbing chromophores.

For oxidatively bleached fabric (Figure 7.6) similar trends were observed as for the unbleached fabric. Sodium hypophosphite treated fabric exhibits a small decrease or no change in visible absorbing chromophores (hence becoming whiter) and a small increase in UV absorbing chromophores, while water, Rongalit C, Blankit D and thiourea dioxide treated fabrics exhibit a decrease in visible (hence becoming whiter) and UV absorbing chromophores in the small to medium range.

The colour of the treated fabrics was quantified using YI and CIE tristimulus values (Tables 7.5 and 7.6). (It is worth noting that the YI and CIE tristimulus values as a whole are a lot lower here than for the UV and blue light treated fabrics (Sections 7.3.1 and 7.3.2) and so an allowance has to be made for that in comparing the effect of the different treatments.) For unbleached fabric (Table 7.5) water, Rongalit C and sodium hypophosphite treated fabrics manifest a small increase in YI (0.21 to 0.48), while Blankit D and thiourea dioxide treated fabrics manifest a large decrease in YI (4.37 and 4.25). The Y-Z values (providing an indication of yellowness) show the same pattern. The Y values (providing an indication of brightness) provided slightly different results. Water and Rongalit C treated fabrics manifest a decrease in Y value, while Blankit D, sodium hypophosphite and thiourea dioxide treated fabrics manifest an increase in Y value, although the values for the water, Rongalit C and sodium hypophosphite treated fabrics are only slightly different from the untreated fabrics.

Solution	Fluorescence		Colour Measurements		
	mean ^a	standard deviation	Yellowness Index	Y	Y - Z
No solution	100.0	2.1	12.87	56.89	2.75
Water	104.7	2.1	13.08	56.37	2.92
Rongalit C	122.8	2.4	13.35	56.82	2.95
Blankit D	128.4	7.4	8.50	60.13	0.71
Sodium hypophosphite	131.2	13.1	13.20	57.07	3.00
Thiourea dioxide	139.4	2.5	8.62	59.86	0.73

^a arbitrary units

Table 7.5 Relative fluorescence intensities (360 nm excitation) and colour measurements of unbleached fabric after being kept in the dark for 24 hours in 2 % solutions of various reducing agents.

For oxidatively bleached fabric (Table 7.6) similar trends are observed as for the unbleached fabric. The trend is exactly the same for YI and Y-Z values but differ slightly in Y value where all the treated fabrics manifest an increase, although again the values for the water, Rongalit C and sodium hypophosphite treated fabrics are only slightly different from the untreated fabric. There is good general agreement between the different colour measurements here: for both unbleached and oxidatively bleached fabrics, Blankit D and thiourea dioxide treatments show a decrease in yellowness i.e. they became whiter together with an increase in brightness, while water, Rongalit C and sodium hypophosphite treatments show little change in yellowness or brightness.

Solution	Fluorescence		Colour Measurements		
	mean ^a	standard deviation	Yellowness Index	Y	Y - Z
No solution	159.2	8.6	7.40	58.52	-0.18
Water	143.7	4.8	7.45	58.99	-0.07
Rongalit C	153.5	15.8	8.58	58.61	0.42
Blankit D	144.3	3.8	5.04	60.85	-1.23
Sodium hypophosphite	144.2	4.4	7.46	58.82	-0.09
Thiourea dioxide	160.2	7.9	4.30	60.27	-1.71

^a arbitrary units

Table 7.6 Relative fluorescence intensities (360 nm excitation) and colour measurements of oxidatively bleached fabric after being kept in the dark for 24 hours in 2% solutions of various reducing agents.

The fluorescence intensities of the fabrics were also determined (Tables 7.5 and 7.6). For unbleached fabric (Table 7.5), water treated fabric manifests no notable change in fluorescence intensity, while Rongalit C, Blankit D, sodium hypophosphite and thiourea dioxide treated fabrics all manifest a small increase in fluorescence intensity. For oxidatively bleached fabric (Table 7.6), water, Rongalit C, Blankit D, sodium hypophosphite and thiourea dioxide treated fabrics all manifest no notable change in fluorescence intensity. There is a general trend that all the treatments cause a small increase in the fluorescence of unbleached fabric, but no change in the fluorescence of oxidatively bleached fabric.

Although a control treatment is needed to establish the degree of whitening due to chemical action alone (compared with the photobleaching effect of the reducing agent plus light) only two investigations have reported using a control [9,10]. Neither of these used any of the reducing agents presently being investigated.

Again there is no correlation between the colour and fluorescence intensities of the treated fabrics. This is very notable for the oxidatively bleached fabric where there are notable changes in the colour but not in the fluorescence intensity.

These results are considered further in the next Section (Section 7.3.4).

7.3.4 Comparison of the Effect of Reducing Agents with Different Wavelengths of Light

In order to establish the degree of whitening due to chemical action of the reducing agents alone, as well as that due to the interaction of light irradiation with reducing agents, the results presented in Tables 7.1-7.6 are summarised in Table 7.7. They are listed according to the reducing agent used. Each light source/reducing agent combination is given a reference number. The fluorescence intensities for each light source are relative to the untreated unbleached fabric. The colour measurement used is based on Yellowness Index values although as discussed in Section 7.3.1 these are not ideal. To overcome the psychological disadvantage of the fabrics used in Section 7.3.3 (effect of reducing agents alone) having inherent lower YI and CIE tristimulus values an arbitrary Yellowness Index has been used. This is similar to the expression of fluorescence intensities, in that the Yellowness Index values for each light source are relative to the untreated unbleached fabric.

It is worth initially making some general comments on the untreated fabrics. The bleached fabric is more fluorescent and whiter as evidenced by a lower YI value and less visible absorbing chromophores especially around 380-450 nm (compare Figures 7.1, 7.3 and 7.5 with Figures 7.2, 7.4 and 7.6 respectively). Additionally, the untreated fabrics show a variation in their fluorescence and colour measurements e.g. the standard deviations for unbleached fabric. This demonstrates the presence of a natural variation in measurements and reiterates that small changes in values are not notable.

Solution (and reference number)	Unbleached Fabric			Oxidatively Bleached Fabric		
		Fluorescence mean ^a standard deviation	Colour % YI ^b		Fluorescence mean ^a standard deviation	Colour % YI ^b
<u>Not irradiated</u>						
UV light (1)		100.0 9.5	100.0		180.5 13.3	71.0
Blue light (2)		100.0 7.4	100.0		172.5 1.2	77.6
In dark (3)		100.0 2.1	100.0		159.2 8.6	57.5
<u>Water</u>						
UV light (11)		95.9 11.9	109.3		122.8 4.5	118.0
Blue light (12)		119.0 3.8	79.4		170.1 5.9	59.3
In dark (13)		104.7 2.1	101.6		143.7 4.8	57.9
<u>Rongalit C</u>						
UV light (21)		45.0 1.1	100.1		61.4 5.9	85.7
Blue light (22)		112.6 6.3	84.7		153.2 11.6	69.4
In dark (23)		122.8 2.4	103.7		153.5 15.8	66.7
<u>Blankit D</u>						
UV light (31)		58.6 4.0	39.1		61.6 7.6	28.6
Blue light (32)		107.7 6.6	56.2		146.4 3.4	40.1
In dark (33)		128.4 7.4	66.0		144.3 3.8	39.2
<u>Sodium hypophosphite</u>						
UV light (41)		108.3 6.2	110.4		150.7 6.6	125.6
Blue light (42)		129.3 4.6	83.0		176.1 6.6	72.3
In dark (43)		131.2 13.1	102.6		144.2 4.4	58.0
<u>Thiourea dioxide</u>						
UV light (51)		93.1 10.3	52.0		96.6 4.1	42.3
Blue light (52)		161.0 10.0	59.4		185.3 15.6	40.5
In dark (53)		139.4 2.5	67.0		160.2 7.9	33.4

^a arbitrary units

^b arbitrary Yellowness Index

Table 7.7 Relative fluorescence intensities (360 nm excitation) and relative Yellowness Index values of unbleached fabric and oxidatively bleached fabric after being exposed to different wavelengths of light for 24 hours in 2% solutions of various reducing agents.

It is also worth commenting on the effect of water alone. This follows on from the extensive work in Chapter 6 where the effect of blue light and UV irradiation on the fluorescence of unbleached wool was investigated. There it was observed that UV irradiation causes photoyellowing with an increase in fluorescence intensity while (Northlight) blue light causes photobleaching with no change in fluorescence intensity. Here (Table 7.7), it was found that (compared with untreated fabric): using UV irradiation, unbleached fabric was photoyellowed ((11) c.f. (1)) with the fluorescence intensity remaining the same ((11) c.f. (1)) (!), while bleached fabric was photoyellowed ((11) c.f. (1)) with the fluorescence intensity decreasing ((11) c.f. (1)). Using blue light, unbleached fabric was photobleached ((12) c.f. (2)) with the fluorescence intensity increasing ((12) c.f. (2)) (!), while bleached fabric was photobleached ((12) c.f. (2)) with the fluorescence intensity remaining the same ((12) c.f. (2)). The colour changes observed are the same as previously observed/published (Chapter 6/Figure 2.11). There are however some differences in the fluorescence intensities of unbleached fabrics. This may well be due to the natural variation in measurements. With bleached fabric UV irradiation causes a decrease in fluorescence intensity as previously published (Section 2.5.2.2).

For each reducing agent the results using light sources are now compared; firstly with the dark sample to find the effect due to the light and secondly (in brackets, in order to avoid confusion), with the relevant water treatment to allow knowledge of the photosensitivity properties (Section 1.4.4) of the reducing agent to be gained:-

The following results were obtained using Rongalit C as the reducing agent: using UV irradiation, unbleached fabric is photobleached ((21) c.f. (23)) (which is opposite to the use of water alone which leads to photoyellowing ((11) c.f. (13))) with the fluorescence intensity decreasing ((21) c.f. (23)) (whereas with water alone there is no change ((11) c.f. (13))), while bleached fabric undergoes photoyellowing ((21) c.f. (23)) (but to a smaller extent than with water alone ((11) c.f. (13))) with a decrease in fluorescence intensity ((21) c.f. (23)) (but to a larger extent than with water alone ((11) c.f. (13))). Using blue light, unbleached fabric is photobleached ((22) c.f. (23)) (but to a smaller extent than with water alone

((12) c.f. (13))) with no change in fluorescence intensity ((22) c.f. (23)) (whereas with water alone there is an increase ((12) c.f. (13))), while bleached fabric photoyellows ((22) c.f. (23)) (which is the same as with water alone ((12) c.f. (13))) with no change in fluorescence intensity ((22) c.f. (23)) (whereas with water alone there is an increase ((12) c.f. (13))). As the bleached fabric used for dark treatments was inherently whiter, then it can be reasonably argued that Rongalit C treatment of bleached fabric with blue light did lead to an improvement in whiteness.

Using Rongalit C solution with blue light irradiation can produce a whiter fabric than initially but this is not due to the Rongalit C as the fabric would have been even whiter if the Rongalit C had been left out. Use of Rongalit C with UV irradiation can still lead to photoyellowing but the magnitude of this is decreased. Hence, Rongalit C is acting as a 'quencher' rather than as a photosensitiser (Section 1.4.4). Where there is photobleaching it is due to the destruction of visible absorbing chromophores rather than to the production of UV absorbing species which exhibit blue fluorescence.

The following results were obtained using Blankit D as the reducing agent: using UV irradiation, unbleached fabric is photobleached ((31) c.f. (33)) (which is opposite to the use of water alone which leads to photoyellowing ((11) c.f. (13))) with the fluorescence intensity decreasing ((31) c.f. (33)) (whereas with water alone there is no change ((11) c.f. (13))), while bleached fabric is photobleached ((31) c.f. (33)) (which is opposite to the use of water alone which leads to photoyellowing ((11) c.f. (13))) with the fluorescence intensity decreasing ((31) c.f. (33)) (but to a greater extent than with water alone ((11) c.f. (13))). Using blue light, unbleached fabric is photobleached ((32) c.f. (33)) (but to a smaller extent than with water alone ((12) c.f. (13))) with the fluorescence intensity decreasing ((32) c.f. (33)) (which is opposite to the use of water alone which leads to an increase ((12) c.f. (13))), while bleached fabric is photoyellowed ((32) c.f. (33)) (which is the same as with water alone ((12) c.f. (13))) with no change in fluorescence intensity ((32) c.f. (33)) (whereas with water alone there is an increase ((12) c.f. (13))). As the bleached fabric used for dark treatments was inherently whiter then it can be reasonably argued that Blankit D treatment with

blue light or UV irradiation did lead to a larger improvement in whiteness than the dark reaction.

Using Blankit D solution with blue light or especially with UV irradiation produces photobleaching. This is due to the effect of the light as well as the Blankit D. Hence, Blankit D is acting as a photosensitiser. The photobleaching is due to the destruction of visible absorbing chromophores rather than to the production of UV absorbing chromophores which exhibit blue fluorescence.

The following results were obtained using sodium hypophosphite as the reducing agent: using UV irradiation, unbleached fabric is photoyellowed ((41) c.f. (43)) (which is the same as with water alone ((11) c.f. (13))) with a decrease in fluorescence intensity ((41) c.f. (43)) (whereas with water alone there is no change ((11) c.f. (13))), while bleached fabric is photoyellowed ((41) c.f. (43)) (but to a greater extent than with water alone ((11) c.f. (13))) with no change in fluorescence intensity ((41) c.f. (43)) (whereas with water alone there is a decrease ((11) c.f. (13))). Using blue light, unbleached fabric is photobleached ((42) c.f. (43)) (but to a smaller extent than with water alone ((12) c.f. (13))) with no change in fluorescence intensity ((42) c.f. (43)) (whereas with water alone there is an increase ((12) c.f. (13))), while bleached fabric is photoyellowed ((42) c.f. (43)) (which is the same as with water alone ((12) c.f. (13))) with an increase in fluorescence intensity ((42) c.f. (43)) (which is the same as with water alone ((12) c.f. (13))). As the bleached fabric used for dark treatments was inherently whiter then it can be reasonably argued that sodium hypophosphite treatment of bleached fabric with blue light did lead to an improvement in whiteness.

Using sodium hypophosphite solution with blue light irradiation can produce a whiter fabric than initially but this is not due to the sodium hypophosphite as the fabric would have been even whiter if the sodium hypophosphite had been left out. Use of sodium hypophosphite with UV irradiation led to photoyellowing of the same magnitude as if the sodium hypophosphite had not been present. Hence, sodium hypophosphite is a 'weaker version' of Rongalit C - acting as a weak 'quencher' rather than as a photosensitiser. Where there is photobleaching it is again due to the destruction of visible absorbing chromophores rather than to the production of UV absorbing species which exhibit blue fluorescence.

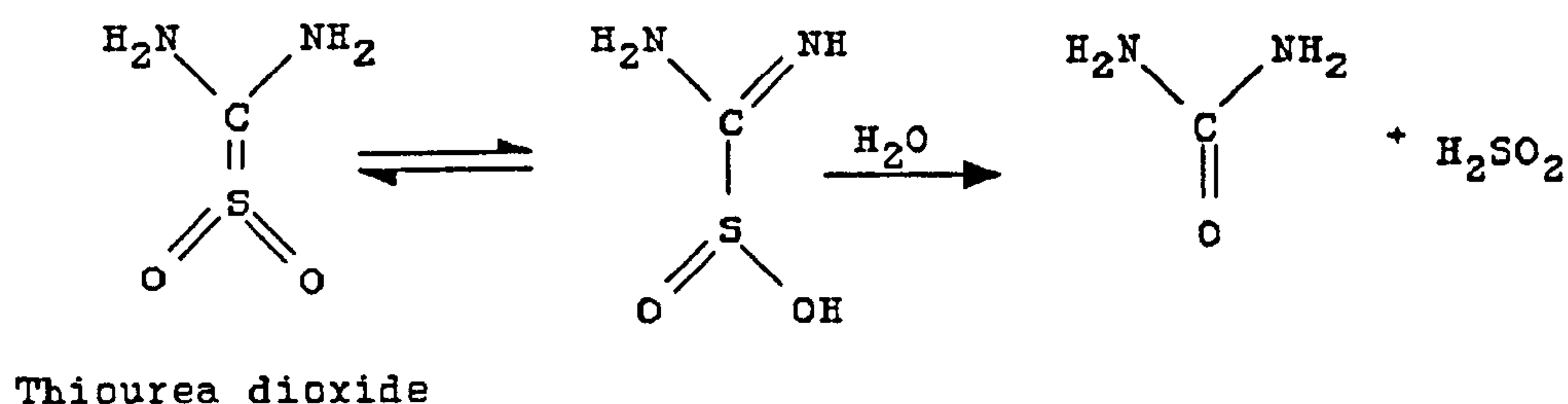
The following results were obtained using thiourea dioxide: using UV irradiation, unbleached fabric is photobleached ((51) c.f. (53)) (which is opposite to the use of water alone which leads to photoyellowing ((11) c.f. (13))) with the fluorescence intensity decreasing ((51) c.f. (53)) (whereas with water alone there is no change ((11) c.f. (13))), while bleached fabric is photoyellowed ((51) c.f. (53)) (which is the same as with water alone ((11) c.f. (13))) with fluorescence intensity decreasing ((51) c.f. (53)) (but to a greater extent than with water alone ((11) c.f. (13))). Using blue light, unbleached fabric is photobleached ((52) c.f. (53)) (but to a greater extent than with water alone ((12) c.f. (13))) with the fluorescence intensity increasing ((52) c.f. (53)) (but to a greater extent than with water alone ((12) c.f. (13))), while bleached fabric is photoyellowed ((52) c.f. (53)) (which is the same as with water alone ((12) c.f. (13))) with no change in fluorescence intensity ((52) c.f. (53)) (whereas with water alone there is an increase ((12) c.f. (13))). As the bleached fabric used for dark treatments was inherently whiter then it can be reasonably argued that thiourea dioxide treatment with blue light or UV irradiation did lead to a larger improvement in whiteness than the dark reaction.

Using thiourea dioxide solution with blue light or UV irradiation produces photobleaching. This is due to the effect of the light as well as the thiourea dioxide. Hence, thiourea dioxide is acting as a photosensitiser. The photobleaching is due to the destruction of visible absorbing chromophores rather than to the production of UV absorbing species which exhibit blue fluorescence, except for blue light irradiation of unbleached fabric which exhibits an increase in blue fluorescence and the destruction of visible absorbing chromophores!

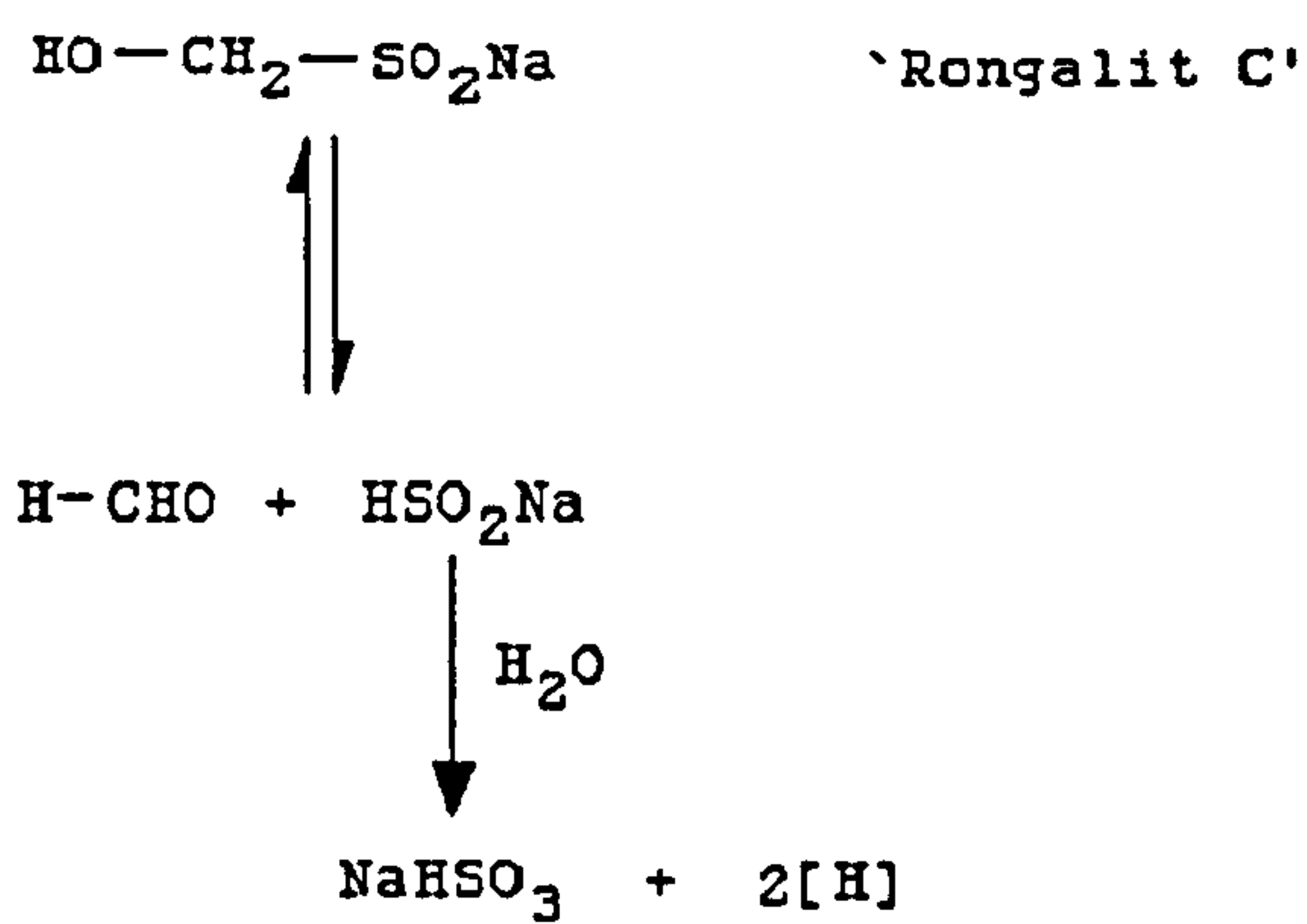
These results can be briefly summarised thus: Rongalit C and sodium hypophosphite act as 'quenchers' - with blue light they retard photobleaching and with UV irradiation they retard photoyellowing. As photosensitisers they are useless. Any improvement in whiteness is due to the destruction of visible absorbing chromophores rather than to the production of UV absorbing species which exhibit blue fluorescence.

Blankit D and thiourea dioxide act as photosensitisers. With both blue light and UV irradiation they produce photobleaching. This is generally due to the destruction of visible absorbing chromophores rather than to the production of UV absorbing species which exhibit blue fluorescence.

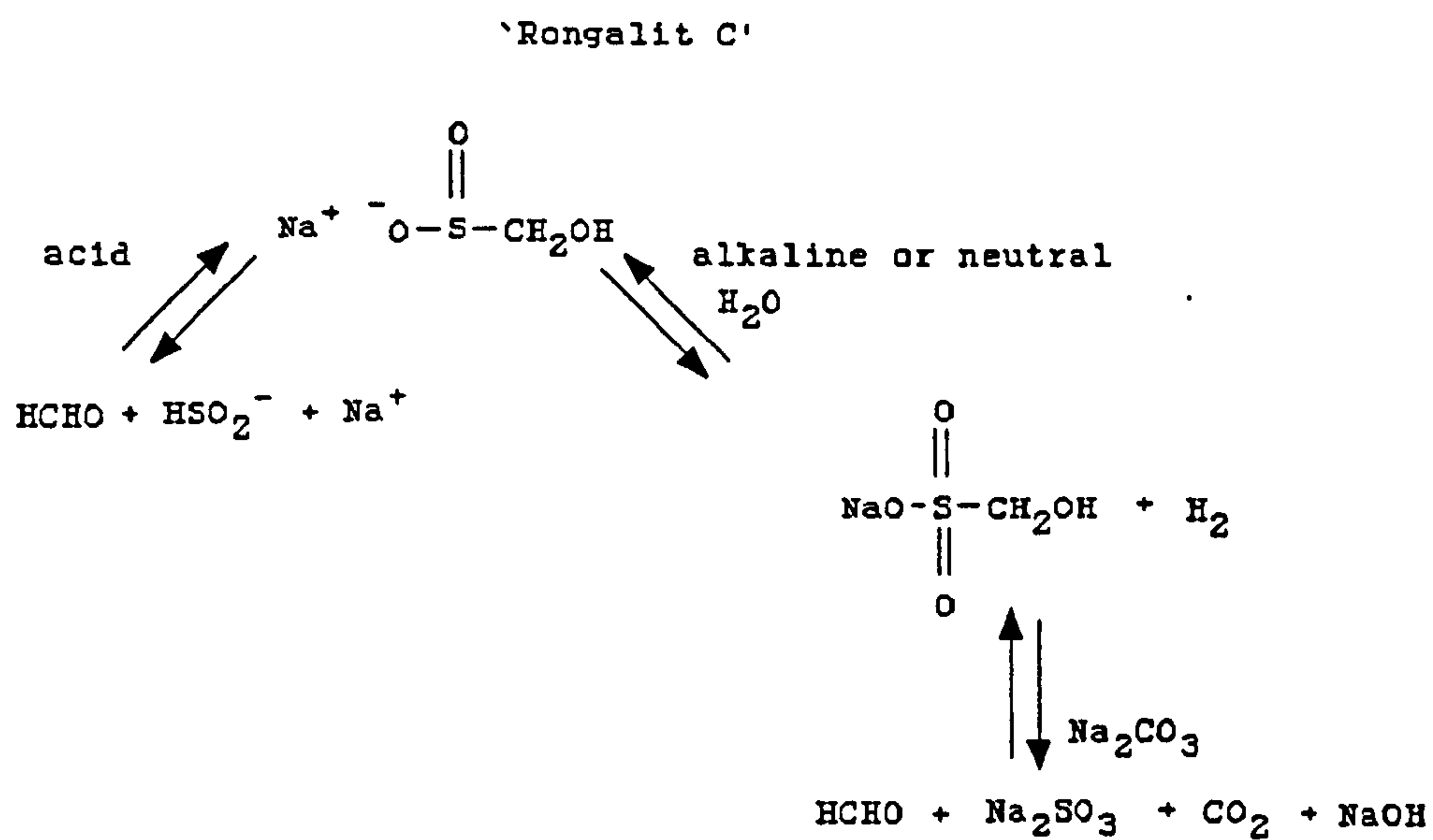
The effects of various reducing agents upon wool having been investigated an understanding of what causes the effects is desirable. This would enable salient points to be capitalised upon. Previous detailed studies, using UV irradiation, have investigated Rongalit C [1,4], Blankit D [1] and thiourea dioxide [4]. In each case photobleaching was observed, unlike the present studies which did not observe notable photobleaching for Rongalit C. Unlike sodium hypophosphite these three are all thought to be a source of sulphinate or sulphinic acid (Schemes 7.1 and 7.2). Sulphinic acid is a powerful reducing agent producing sulphur dioxide on oxidation, which in solution also acts as a reducing agent [4]. The poor quality of Rongalit C as a photosensitiser of photobleaching as compared to Blankit D was explained by Jones in terms of a pH effect (Scheme 7.3) [1]. This shows the dissociation/decomposition pathways of hydroxymethane sulphinates. Jones decided there would be at least partial oxidation of the sulphinate to the sulphonate under the alkaline conditions of Rongalit C (pH 8.0) and that the partial activity of Rongalit C may be due to the presence of a small percentage of formaldehyde and sodium sulphinate. An attempt to acidify a solution of Rongalit C failed as the reagent immediately precipitated out. Jones thus decided that although Rongalit C and Blankit D are structurally similar their reactivity with wool is different.



Scheme 7.1 [4]



Scheme 7.2 [4]



Scheme 7.3 [1]

The pH of the 2% solutions of reducing agents used in the present study were measured and found to be:

Water	7
Rongalit C	10
Blankit D	3
Sodium hypophosphite	9
Thiourea dioxide	4

The solutions of the two reducing agents which act as photosensitisers, Blankit D and thiourea dioxide, are both acidic, while the solutions of the two reducing agents which did not act as photosensitisers, Rongalit C and sodium hypophosphite, are both alkaline. Clearly pH plays an important part in the reducing agent assisted photobleaching of wool.

Incidentally Jones investigated the role of the 20% stabilisers and complexing agents which are present in Blankit D in the presence of the 80% zinc formaldehyde sulfoxylate (c.f. Section 7.2) and decided that the stabilisers only promote the whitening process by prevention of the decomposition of the reducing agent, rather than by reacting themselves with the wool [1].

7.4 CONCLUSION

The effect of four reducing agents on wool has been investigated - in the presence of UV irradiation, blue light irradiation and in the dark. It is important to run blank experiments as often the reducing agents have an effect on their own. The ones investigated fell into two groups.

The first group, consisting of Rongalit C and sodium hypophosphite, had little effect on the colour or the fluorescence of the wool on their own. In the presence of UV irradiation or blue light they retard the effect of light - reducing the amount of photoyellowing on UV irradiation and the amount of photobleaching on blue light irradiation.

The second group, consisting of Blankit D and thiourea dioxide, had an effect on their own, causing a whitening of the wool but with little effect on the fluorescence. In the presence of UV irradiation or blue light irradiation they act as photosensitisers causing extensive photobleaching.

All the whitening observed was due to the destruction of visible absorbing chromophores rather than to the production of UV absorbing chromophores which exhibit blue fluorescence, with one exception. Unbleached fabric irradiated with blue light in the presence of thiourea dioxide experienced a destruction of visible absorbing chromophores and an increase in blue fluorescence! For the other treatments, rather than the fluorescence remaining the same many actually caused the fluorescence to decrease.

The difference between the two groups is attributed to the pH of the solutions used, with acidic conditions sensitising photobleaching. Further work, using a larger range of reducing agents, is required to establish how general this is. The photostability of the whitened fabrics obtained from the reducing agent assisted photobleaching could also be usefully investigated.

7.5 REFERENCES

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CHAPTER 8

NATURAL FLUORESCENCE OF

WOOL YOLK/GREASE

8.1 INTRODUCTION

In this chapter the nomenclature of Truter [1] is adhered to, as described in Section 3.1.

A large amount of research has been undertaken in order to identify the components of wool wax (Section 3.2.1). Surprisingly, very little work has been performed investigating the natural fluorescence of wool wax/grease (Section 3.2.4). In fact only five previous reports [2-6] even make mention of this fluorescence including one which denies its existence [3]. Additionally, only one [6] makes anything other than brief comments on the subject.

Whilst performing preliminary fluorescence microscopy investigations for the work in Chapter 5 the issue of the fluorescence of wool wax was raised.

This chapter describes a series of experiments which have been carried out in order to observe the natural fluorescence of the wool yolk along a wool fibre, characterise the spectral distribution of the fluorescence of wool grease, attempt to identify the components of wool grease causing the fluorescence and to observe the effect of light irradiation upon the fluorescence intensity.

8.2 EXPERIMENTAL

A detailed explanation of the main materials and techniques used can be found in Chapter 4.

8.2.1 Variation in Natural Fluorescence Along Fibres

Fibres from Merino fleece 2 (average diameter $21.5\mu\text{m}$) were used without being cleaned. Snippets were obtained from the staples of tip ends, middle portions and root ends (c.f. Section 5.2.1). Cross sections were prepared using a Hardy microtome and photomicrographs obtained using both UV and violet excitation modes of a M17 fluorescence microscope using a 25/0.5 objective (Section 4.4.2.1). Camera exposure times of 18 seconds were used (Kodachrome 64 ASA Professional (Daylight) colour transparency film).

8.2.2 Spectral Characterisation

8.2.2.1 Choice of Solvent

The solubility of wool grease (Merino wool grease) was investigated using 26 solvents of various grades which were readily available. They were found to divide into three general groups (Table 8.1).

For preliminary fluorescence measurements (and those in Section 8.3.3) solutions of wool grease in dichloromethane were used. An appreciation of the fluorescence quenching properties of chlorinated solvents through the heavy atom effect (see Section 1.4.4) led to this solvent (and other chlorinated solvents) no longer being used. After considering the remaining grease-soluble solvents for toxicity, availability in spectroscopic grade, on site availability and price of replacement, cyclohexane was chosen. It is partly an arbitrary choice as it is not the only suitable solvent.

Insoluble	Partly Soluble	Soluble
acetone acetonitrile dioxane dimethyl- sulphoxide ethanol methanol propan-2-ol	diethyl ether diethylene glycol dimethylformamide ethyl acetate n-hexane 'hexane fraction' n-heptane 60-80°C petroleum ether	carbon tetrachloride chloroform dichloromethane tetrachloroethene benzene t-butanol cyclohexane methyl cyclohexane 100-120°C petroleum ether tetrahydrofuran toluene

Table 8.1 The solubility properties of Merino wool grease in a number of solvents.

8.2.2.2 Determination

The fluorescence intensities of the wool greases W1-W6 and Merino wool grease were obtained using a MPF-4 fluorescence spectrophotometer (Section 4.4.1). The wool greases were dissolved in cyclohexane (Spectroscopic grade, BDH) and made to give an optical density of 0.10 at 350 nm (Section 4.5.3), before being flushed with argon for 10 minutes (Merino wool grease 20 minutes). The samples were sequentially excited at 300, 350, 400, 450 and 500 nm and for each excitation wavelength the fluorescence emission spectra were recorded.

UV/visible absorption spectra and infra-red spectra of the wool greases W1-W6 were also obtained.

8.2.3 An Attempt to Isolate the Species Responsible for Fluorescence

Preliminary investigations were carried out using silica thin layer chromatography (tlc) sheets (layer thickness 0.2 mm, DE Plastik folien Kieselgel 60 F₂₅₄ (Merck)). Merino wool grease was dissolved in chloroform and tlc performed using hexane/ethyl acetate in a variety of ratios. The ratio 4:1 was found to provide a reasonable separation.

Quantitative investigations were then carried out using preparatory tlc plates (20 cm x 20 cm, silica, obtained from Mr T. Gilby, City University). Merino wool grease was dissolved in chloroform and applied to the plates prior to elution with hexane/ethyl acetate (4:1). Two plates were run: on the first 98.9 mg of wool grease was used and on the second 107.1 mg. After elution the fractions from the two plates were combined.

The fractions were located using a 254 nm tlc lamp. The fractions were not completely separated from one another so overlapping portions were discarded. The fractions were removed, washed with dichloromethane/ethyl acetate (1:1, 3x approximately 15 ml) using a sintered glass frit and the resulting solutions evaporated under reduced pressure. This was followed by dissolving in dichloromethane, transfer to a weighed sample bottle, evaporation of the solvent using a stream of nitrogen and the sample bottle being reweighed.

The fluorescence intensities of the fractions and Merino wool grease were obtained using a MPF-4 fluorescence spectrophotometer (Section 4.4.1). The samples were dissolved in dichloromethane (Spectroscopic grade) and made to give an optical density of 0.10 at 350 nm (but without correction for solvent absorbance) (at the same time UV/visible absorption spectra were obtained). The samples were sequentially excited at 300, 350, 400 and 450 nm and for each excitation wavelength the fluorescence emission spectra were recorded. Some samples were also excited at 500 nm.

8.2.4 Effect of Light Irradiation

8.2.4.1 Effect of Blacklight (UV) Irradiation

W1 and Merino wool grease were each placed on a glass plate (approximately 20 x 5 cm) which had been warmed and were spread evenly over most of the plate using a spatula. The greases were then irradiated using Irradiation Method 1 equipped with Blacklight lamps (Philips, 3 x 18W, 600 mm) and were periodically sampled.

The fluorescence intensities of the samples were obtained using a MPF-4 fluorescence spectrophotometer (Section 4.4.1). The samples were dissolved in cyclohexane (Spectroscopic grade, BDH) and made to give an optical density of 0.10 at 350 nm (at the same time UV/visible absorption spectra were obtained) before being flushed with argon for 10 minutes. The samples were excited at 350 nm and the fluorescence emission between 360 and 620 nm was measured and taken as indicative of the fluorescence arising from the 'grease'. The samples were also excited at 400 nm and the fluorescence emission between 630 and 710 nm was measured and taken as indicative of the fluorescence arising from chlorophyll in the wool grease.

8.2.4.2 Effect of *In Situ* Irradiation

W1 was placed on a glass slide and irradiated *in situ* on the LAB16 microscope, equipped with a 16/0.40 Neofluar objective, for 3 min and then for an additional 5 min (Irradiation Method 4). The fluorescence spectrum of the sample was measured initially, after 3 min irradiation and again after 8 min irradiation. The investigation was performed twice - once using UV excitation mode and once using visible excitation mode.

Croda wool grease was placed on a glass slide and irradiated *in situ* on the LAB16 microscope, equipped with a 40/0.75 Neofluar objective for 30 sec using an argon ion laser (Irradiation Method 5). The fluorescence intensity at various wavelengths was measured, before and after irradiation, using the argon ion laser (pulse length 1 μ s) and the visible mode of the microscope.

8.3 RESULTS AND DISCUSSION

8.3.1 Variation in Natural Fluorescence Along Fibres

Cross sections of tip, middle and root portions of uncleaned Merino staples were prepared and fluorescence micrographs produced using both UV and violet excitation modes. The most intense fluorescence was obtained using the violet excitation mode and therefore only these results are reproduced in Figure 8.1 (a-c).

The cross section of the root portions (Figure 8.1 (a)) shows practically zero emission from the fibres along with an intense fluorescence from the wool yolk surrounding the fibres. This effect is not uniform as there appears to be intense yolk fluorescence from some of the fibres. This is probably due to the scalpel used in preparing the cross section (Section 4.4.2.1) inadvertently spreading the yolk as it cut through the fibres.

Some spreading of the yolk is also observed in the cross section of the middle portions (Figure 8.1 (b)). The fibres have a weak fluorescence. However, the wool yolk has a bright fluorescence but less than the root portions. In the cross section of the tip portions (Figure 8.1 (c)) only a medium fluorescence from the fibres can be seen!

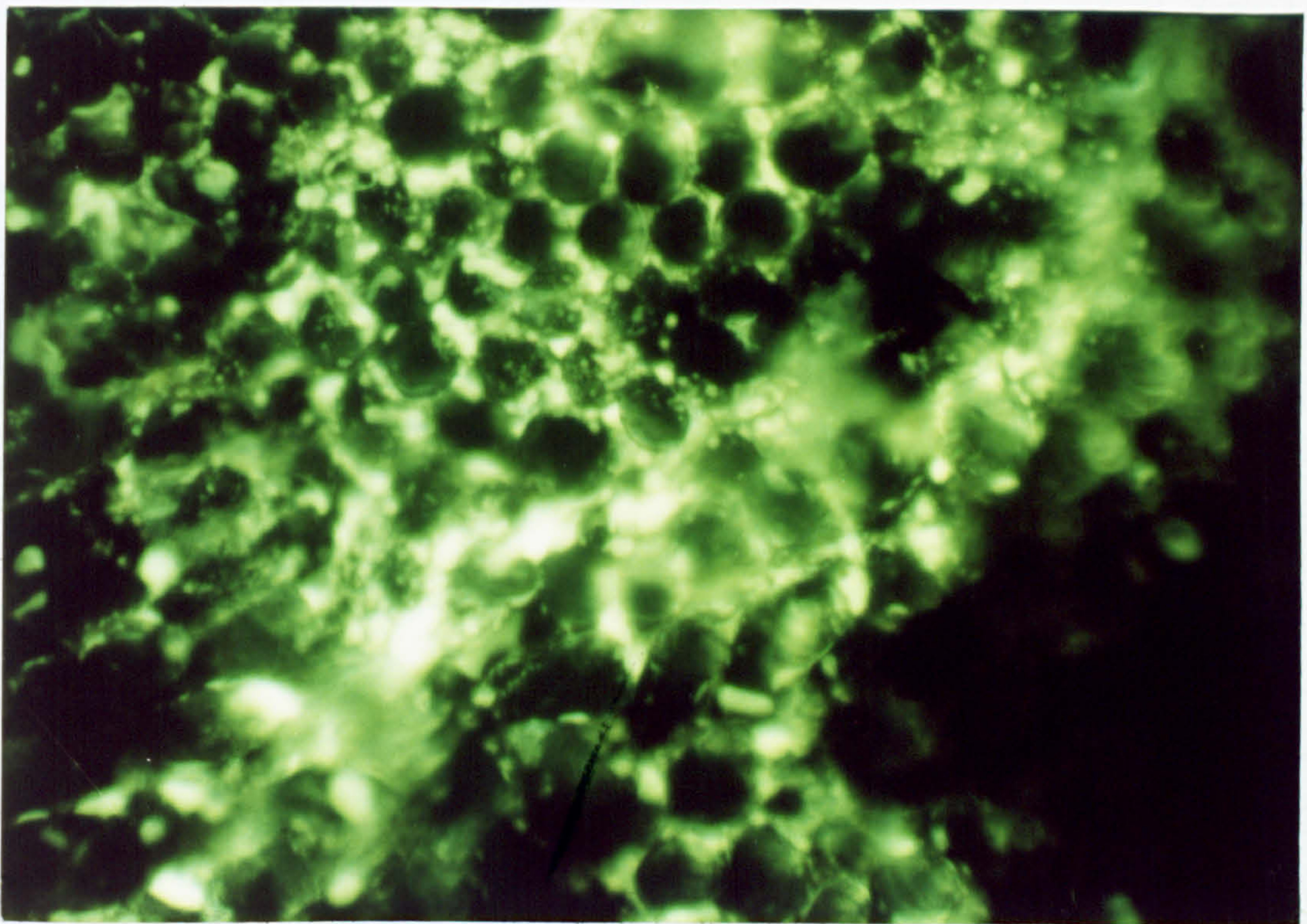


Figure 8.1 (a) Fluorescence micrograph of a cross section of the root region of an uncleaned Merino wool staple (violet excitation, magnification x360).

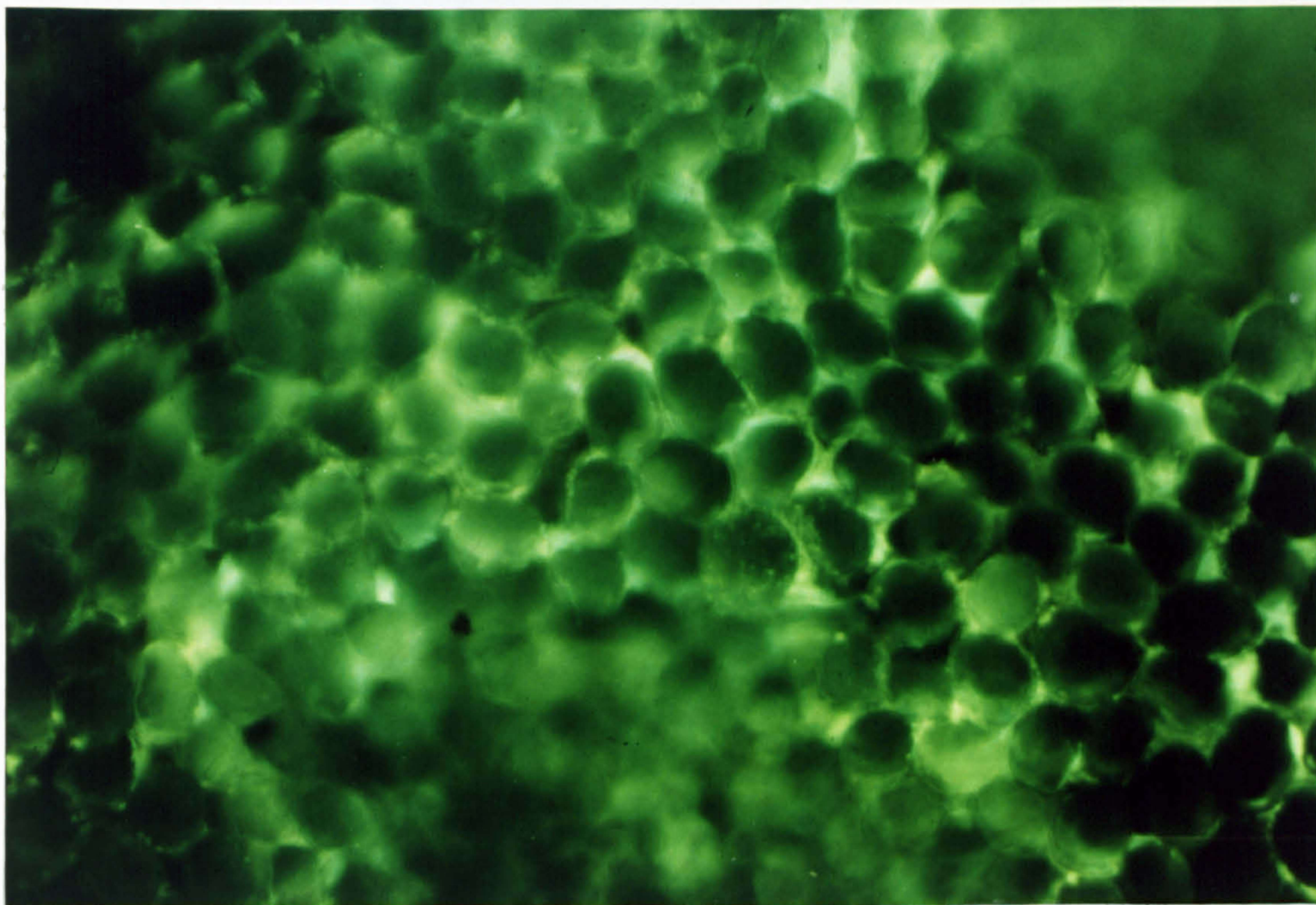


Figure 8.1 (b) Fluorescence micrograph of a cross section of the middle region of an uncleaned Merino wool staple (violet excitation, magnification x360).

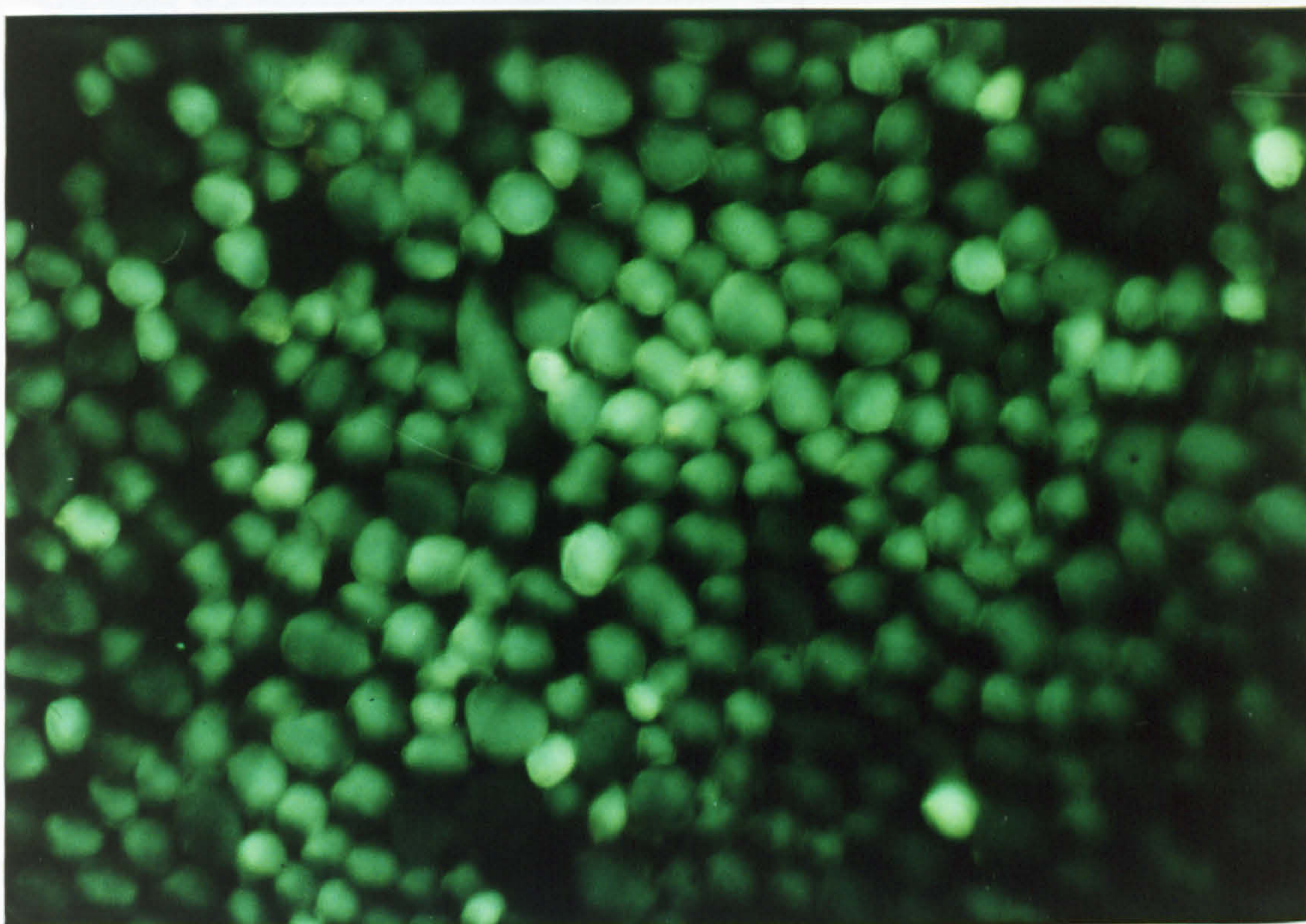


Figure 8.1 (c) Fluorescence micrograph of a cross section of the tip region of an uncleaned Merino wool staple (violet excitation, magnification x360).

As far as the wool fibres are concerned this agrees with the previous finding (Section 5.3.1 - Figures 5.1 and 5.3) that the natural visible fluorescence of Merino fibres varies along the length of the fibre, from highly fluorescent tips to barely fluorescent roots. In comparing Figures 5.1 and 8.1 it should be borne in mind that different (constant) exposure times were used for each set.

Hirst [3] while describing the appearance of some textiles in UV radiation reported that 'clipped wool Australian merino greasy' gave a 'Yellow colour with bluish-white tips'. This confirms the findings of Figure 8.1 and raises the question as to why there is no wool yolk fluorescence from the tip portions. There are a number of possible explanations. Firstly, the yolk could have become non-fluorescent as a result of the natural weathering process. However, studies on the effect of UV and blue light irradiation on wool grease (Section 8.3.4) revealed that although the natural fluorescence decreased greatly over a long period of time it did not reach zero in the time investigated. Secondly, the yolk could have been removed by the natural weathering process. It is known that weathering of wool fat/lanolin leads to an increase in water soluble substances (Section 3.2.2) which could have been lost from the fleece by rain before the sheep was sheared. It is hard to imagine that this would account for the difference on its own. Thirdly, and more likely, the explanation could be a combination of the first two coupled with the sensitivity of the film (or in the case of Hirst, the eye) i.e. as the yolk weathered over a long period of time some was lost from the fibre and the fluorescence of the remaining decreased to such a point that it did not register on the film (or eye). Further work needs to be carried out before this explanation can be accepted unequivocally.

Fluorescence micrographs (not shown) were also obtained of longitudinal portions of uncleaned Merino fibres. These revealed that the wool yolk was present in the form of patches and 'blobs' rather than being uniformly spread over the fibre.

The possibility exists that some of the fluorescence of the yolk arises from the presence of biodeteriogens. Spores of these bacteria and fungi are universal, only needing suitable conditions to develop [7]. Wool stained by the action of biodeteriogens sometimes produce fluorescence when the material is examined under UV light [3,8-11]. Not only can biodeteriogens be fluorescent themselves [11,12] but they are also capable of producing fluorescent pigments (Section 3.3.1).

Not all sheep are affected the same way. They divide into two groups - immune and susceptible sheep [13]. (Selective breeding is the best way to reduce the incidence of susceptible sheep [7,13].) It has been suggested that in susceptible fleeces, warmth and moisture may induce wax breakdown through detergent action of suint [7]. Additionally ester-splitting of wool wax in fleece-rot and mycotic dermatitis has also been suggested [14]. However, some samples of wool wax show a high inhibitory effect on the actinomycete *Dermatophilus congolensis* (the organism responsible for the disease in sheep known as 'lumpy wool') [15]. The activity of the wax was attributed to the two saturated fatty acids (+) 10-methyldodecanoic acid and 12-methyltridecanoic acid which were obtained after saponification, and which were found to have high inhibitory activity against Gram positive bacteria.

Significant microbial spoilage may be readily apparent due to severe pigmentation and fibre tendering; while a characteristic fusty odour is normally associated with fungal growth [7]. None of these attributes were apparent with the wool fleece used to prepare the fluorescence micrographs of Figures 8.1 (a-c). Additionally, it has been reported that direct microscopic examination may reveal extensive fibre damage due to bacterial activity (normally identified by the characteristic production of cortical cells) or fungal growth on the surface or within the fibre [7]. Similarly, these properties were not readily apparent with the wool fleece used. Consequently, biodeteriogens did not contribute significantly, if at all, to the fluorescence of the yolk in Figures 8.1 (a-c).

Yolk consists of two main fractions: wool wax and suint (Section 3.1). It is not apparent from the fluorescence micrographs whether the fluorescence is restricted to only one of the fractions. The fluorescence of wool wax is considered in the next section (Section 8.3.2). Further work investigating the fluorescence properties of suint could usefully be carried out.

These results confirm that wool yolk is (highly) fluorescent and shows that the amount of wool yolk/intensity of wool yolk fluorescence varies along a Merino wool fibre.

8.3.2 Spectral Characterisation

An attempt was made to recover wool wax from Merino wool (Section 4.2.2) but this was not particularly successful as it proved difficult to remove the dirt from the wax. Consequently, the rest of the studies in this chapter involve the use of commercial wool grease.

Although it has been known for a long time that wool grease fluoresces (Section 3.2.4) the fluorescence spectrum of wool grease has not been reported. Figure 8.2 shows the fluorescence spectra of a commercial Merino wool grease for a number of excitation wavelengths. (Figures 8.2 and 8.3 have been redrawn from the originals by taking measurements every 5 nm and have been corrected to remove the Raman peaks arising from the solvent, cyclohexane.) The presence of a number of emission maxima (see also Table 8.2) indicates the presence of more than one chromophore in the wool grease and has resemblances of the situation reported for wool (Section 2.5.2.1, especially Figures 2.13 and 2.14 as well as [16]).

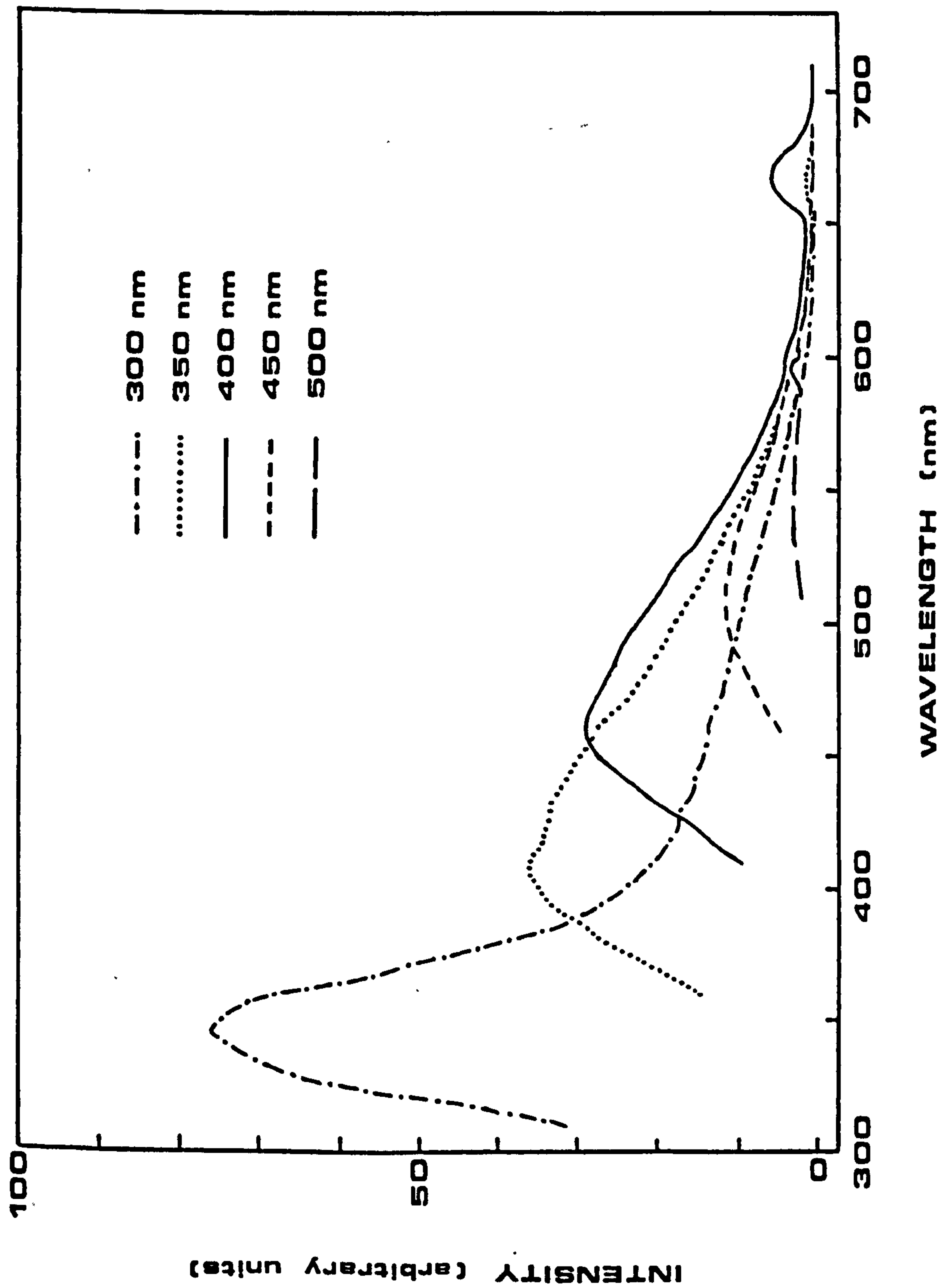


Figure 8.2 Fluorescence spectra of Merino wool grease for a number of excitation wavelengths.

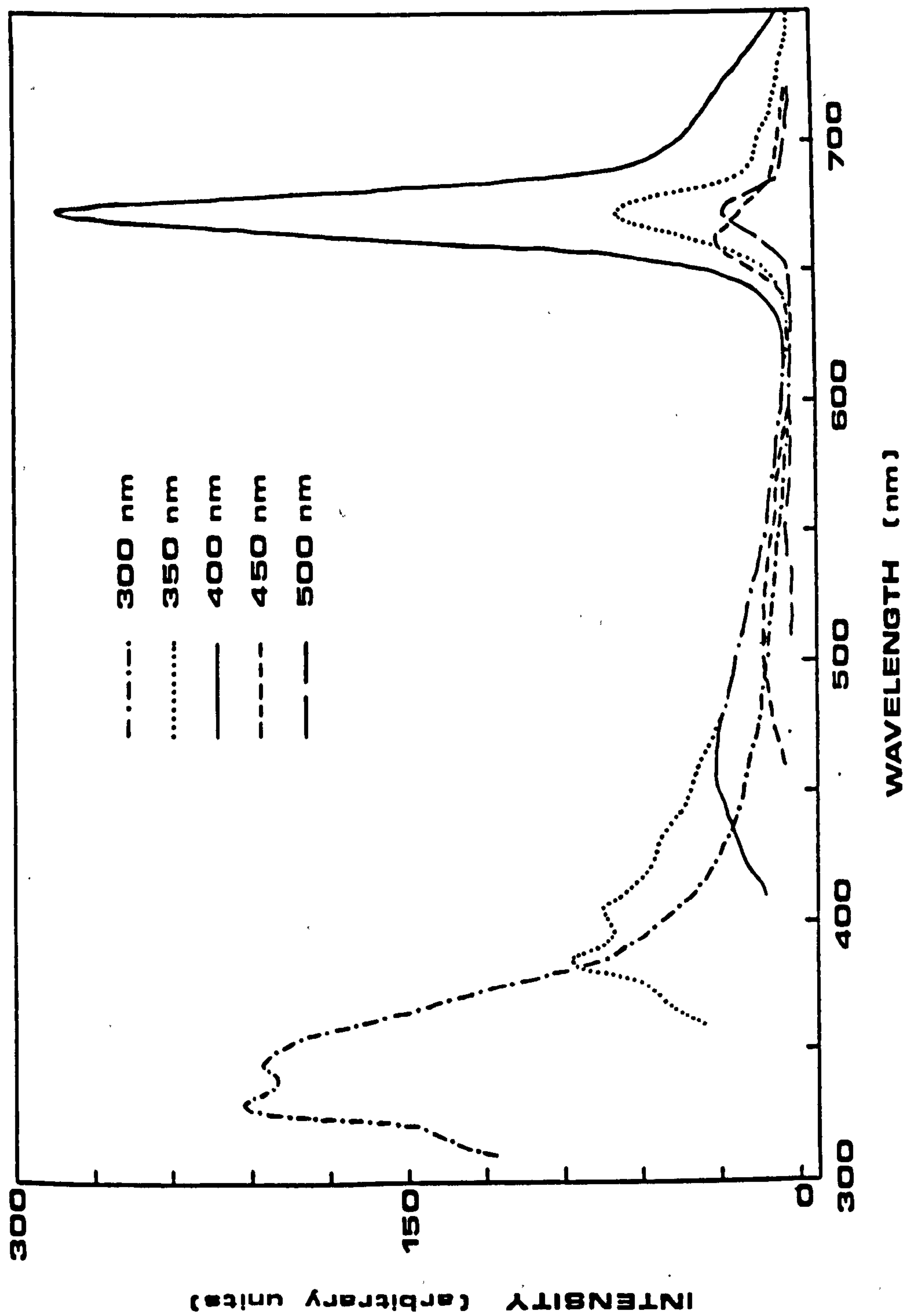


Figure 8.3 Fluorescence spectra of W1 for a number of excitation wavelengths.

EW ^a (nm)	Fluorescence Emission Maxima (nm) and Fluorescence Intensities ^b		
	Merino	W1	W2
300	345(76)	330(213), 345(206)	355(97)
350	405(36)	385(86), 405(75), 672(69)	405(63), 665(4)
400	460(29), 665(6)	455-465(31), 672(283)	460(29), 665(17)
450	510(12)	510-525(13), 660(30)	510-520(9), 660(2)
500	530-560(3)	540-560(4), 672(27)	535-560(2), 665(1)

^a excitation wavelength

^b intensities measured at peak height and expressed in arbitrary units

Table 8.2 Fluorescence emission maxima and relative fluorescence intensities of three wool greases after excitation at a number of wavelengths.

Figure 8.3 shows the fluorescence spectra of W1 for a number of excitation wavelengths (W1 is a New Zealand wool grease containing heavy chlorophyll contamination, which was obtained from the bulk of a small drum (Section 4.2.2)). Again, the presence of a number of emission maxima (see also Table 8.2) indicates the presence of more than one chromophore in the wool grease. Very noticeably for this wool grease is a peak at 672 nm. As this wool grease is known to be heavily contaminated with chlorophyll (Section 4.2.2) then it could be due to chlorophyll. Chlorophyll a in toluene fluoresces with an emission maximum at 670 nm (Section 9.2.1) indicating that the emission peak at 672 nm in wool grease spectra is due to chlorophyll a. This could have arisen from dung contamination (Section 4.2.2 and c.f. Section 2.4.6) or possibly by contact with plants directly. Higher plants contain chlorophyll a and chlorophyll b (in the ratio 3:1) [17]. In diethyl ether chlorophyll a fluoresces with a maximum at 669 nm while chlorophyll b fluoresces with a maximum at lower wavelength, 647 nm [18]. The fluorescence emission peak at 660 nm in wool grease would seem to be due to chlorophyll b.

The fluorescence spectra of W2 for a number of excitation wavelengths were also recorded (not shown). (W2 is a New Zealand wool grease which was obtained from the same drum as W1 but from the top rather than from the bulk of the drum (Section 4.2.2).) These spectra are similar to those for the Merino and W1 wool greases with the presence of a number of (similar) emission maxima (Table 8.2).

Noticeable though is the much reduced intensity of the chlorophyll emission peaks, along with the reduced intensity of the ‘grease’ peaks especially that produced upon excitation at 300 nm. Closer examination of the fluorescence spectra of Merino wool grease (Figure 8.2) reveals the presence of a small amount of chlorophyll a. The intensities of the spectra from the Merino wool grease are similar to those from W2.

Wool Grease	Fluorescence Intensity at: ^a	
	350 nm ^b	670 nm ^c
Merino	75	6
W1	201	282
W2	97	17
W3	105	56
W4	40	5
W5	171	89
W6	64	14

- ^a arbitrary units (the same as Table 8.1)
- ^b peak height at 350 nm after exciting 300 nm
- ^c peak height at 670 nm after exciting at 400nm.

Table 8.3 Fluorescence intensities of a number of wool greases after excitation at a number of wavelengths.

The fluorescence spectra of W3 and W4 (being the bulk and top, respectively, of a small drum of a New Zealand wool grease containing lesser chlorophyll contamination (Section 4.2.2)) as well as W5 and W6 (being the bulk and top, respectively, of a small drum of a typical New Zealand wool grease (Section 4.2.2)) were obtained (not shown). Like W2 these spectra are similar to those for the Merino and W1 wool greases with the presence of a number of (similar) emission maxima. The results of the Merino and W1-W6 wool grease samples are briefly summarised in Table 8.3. The fluorescence intensity at 350 nm is an approximate measure of the amount of ‘grease’ fluorescence, while the fluorescence intensity at 670 nm is an approximate measure of chlorophyll

fluorescence. It is clear that all the wool greases have at least some chlorophyll in them. For the New Zealand wool greases the top of the drums (W2, W4 and W6) have a lower level of 'grease' fluorescence together with a much lower level of chlorophyll fluorescence than the bulk of the drums (W1, W3 and W5 respectively).

The infra-red spectra (not shown) of the wool greases from the top of the drums (W2, W4 and W6) showed (relative to their parent wool greases: W1, W3 and W5 respectively) an increase in absorption in the range $1400-1000\text{ cm}^{-1}$ and a large increase in absorption at 1676 and 1550 cm^{-1} . It is known that weathering causes an increase in absorption at $1670/1680\text{ cm}^{-1}$ corresponding to $\alpha\beta$ -unsaturated ketones (Section 3.2.2). This agrees with the supplier's observation (Section 4.2.2) that the wool greases at the top of the drums had undergone appreciable oxidation, resulting in them being considerably lighter, tackier and harder than their parent wool greases. The decrease in chlorophyll fluorescence on weathering of the wool greases (top of the drums relative to the bulk) is not surprising as chlorophyll is known to be light sensitive [17,19].

Thus, it seems clear that a new measure of weathering of wool grease is the decrease in the level of fluorescence intensity and this suggests that the Merino wool grease examined had already undergone weathering. However, care has to be taken on this last point. The Merino wool grease presumably came from Australian sheep while wool greases W1-W6 did come from New Zealand and the climatic conditions may be different, resulting in a different amount of chlorophyll contamination in the first place. Although, even if this is correct, it does not, *per se*, account for the lower level of 'grease' fluorescence which the Merino wool grease exhibited.

To reiterate, the fluorescence spectra of a number of wool greases were obtained using a range of excitation wavelengths. These revealed the presence of a number of chromophores, of which two, chlorophyll a and chlorophyll b, have been readily identified. Weathering results in a decrease in the fluorescence intensity arising from both the main 'grease' and also the chlorophyll contamination.

8.3.3 An Attempt to Isolate the Species Responsible for Fluorescence

The previous section revealed the presence of a number of chromophores in wool grease which fell into two groups. The first consisted of chlorophyll a and chlorophyll b and the second of a number of 'grease' components. In this section an attempt is made to separate wool grease into its various components in order to establish which of these 'grease' components fluoresce and what their identities are.

Commercial Merino wool grease was separated using preparatory tlc. Nine fractions (identified by using a 254 nm tlc lamp) were recovered (Table 8.4). From applying 206.0 mg of wool grease, 124.5 mg (60%) was recovered in these nine fractions, overlapping portions having been discarded (and some having been split in the recovery process). The fractions varied in qualitative fluorescence intensity (as just mentioned) and in colour indicating a number of constituents. By way of comparison the colour of unseparated Merino wool grease is yellowy brown.

Fraction Number	Rf ^a	Fluorescence Intensity ^b	Mass (mg)	Colour
1	0.94	medium	35.4 ^c	pale yellow waxy
2	0.84	strong	3.5 ^c	solid ^d
3	0.80	medium	14.1	light brown
4	0.73	medium	22.5	brown
5	0.61	weak	11.0	golden yellow waxy
6	0.42	weak	4.2	yellowy/brown
7	^e	^f	11.0	golden yellow waxy
8	0.12	medium	5.4	dark brown
9	0	strong	17.4	dark brown

^a ratio of the distance travelled by the solute to the distance travelled by the solvent. ^b as observed using a 254 nm tlc lamp.

^c amount remaining after some having been split.

^d too small an amount to see the colour.

^e all on the tlc plates between fractions 6 and 8. ^f non-fluorescent.

Table 8.4 Various pieces of information concerning the fractions obtained from the tlc separation of Merino wool grease.

The UV/visible absorption spectra of the fractions reveal that most have a very broad UV absorption like the parent wool grease (Table 8.5). This indicates that the fractions are still mixtures. Two of the fractions (numbers 2 and 9) exhibit a shoulder in their UV absorbance indicating slightly more specificity, while only one fraction (number 8) has a definite peak and yet even this is superimposed onto a broad UV absorbance.

Fraction Number	UV/vis Absorption	Approximate Fluorescence Emission Maxima (nm) and Intensities for Given Excitation Wavelengths (nm) ^a				
		300	350	400	450	500
Merino	v. bd ^b UV	355(21)	410(27)	460(33)	510(18)	^c
1	v. bd ^b UV	340(159)	380(63)	430(42)	515(15)	^c
2	v. bd ^b UV ^d	350(49)	390(47)	455(41)	520(70)	540(47)
3	v. bd ^b UV	350(123)	395(87)	435(48)	520(24)	broad(3)
4	v. bd ^b UV	345(64)	395(47)	460(43)	505(34)	550(9)
5	v. bd ^b UV	350(69)	405(66)	450(63)	510(29)	560(12)
6	v. bd ^b UV	345(117)	400(108)	440(57)	515(27)	^c
7	v. bd ^b UV	340(60)	405(58)	445(60)	500(30)	540(11)
8	bd ^e	350(23)	395(35)	460(55)	515(63)	535(59)
9	bd ^f	360(17)	425(38)	455(71)	510(69)	535(57)

^a intensities measured at peak height and expressed in arbitrary units.

^b very broad UV. ^c not recorded.

^d small shoulder at 320 nm. ^e broad with peak at 274 nm.

^f broad with shoulder at 260 nm.

Table 8.5 UV/visible absorption peaks as well as approximate fluorescence emission maxima and relative fluorescence intensities after excitation at a number of wavelengths, of Merino wool grease and fractions obtained from it.

Fluorescence spectra of Merino wool grease as well as the nine fractions were recorded for a number of excitation wavelengths (not shown). These spectra are similar to the 'grease' component of the spectra already reported (Figures 8.2 and 8.3) with the presence of a number of emission maxima (Table 8.5) (the values in Table 8.5 have been corrected to remove gross Raman effects only (notably the peak at 330 nm on excitation at 300 nm) and do not set out to cover the chlorophyll part of the spectra).

Each fraction is clearly still a mixture although there are some differences between the fractions in their relative intensities at the different exciting wavelengths, and also some (small) differences in their emission maxima. The latter observation may not be significant given that many peaks are broad.

In Section 3.2.1.1 the limited amount of work reported on identifying the esters in wool wax was described. It was shown that only about 50 esters had been identified and reported a calculation that wool wax must contain at least 8000 mono- and diesters, in addition to polyesters [1]. Consequently, it is not surprising that the preparatory tlc described has failed to isolate individual components present in wool grease. This work has however shown that the phenomenon of components in wool grease to fluoresce is widespread. Further work on the isolation of wool grease components to establish which components fluoresce and to find out their identity could usefully be carried out.

8.3.4 Effect of Light Irradiation

8.3.4.1 Effect of Blacklight (UV) Irradiation

The effect of UV irradiation on the natural fluorescence of wool wax was investigated by irradiating two types of wool grease with Blacklight fluorescent lamps. Both Merino wool grease (Figure 8.4) and W1, a heavily pigmented New Zealand wool grease, (Figure 8.5) were used. In each case the fluorescence arising from excitation at 350 nm was taken as indicative of the 'grease' fluorescence while the fluorescence arising from excitation at 400 nm was taken as indicative of chlorophyll fluorescence (Section 8.2.4.1, c.f. Figure 8.3). For each wool grease the intensities of the 'grease' and chlorophyll fluorescence are relative to the initial intensity of the 'grease' fluorescence (at day 0).

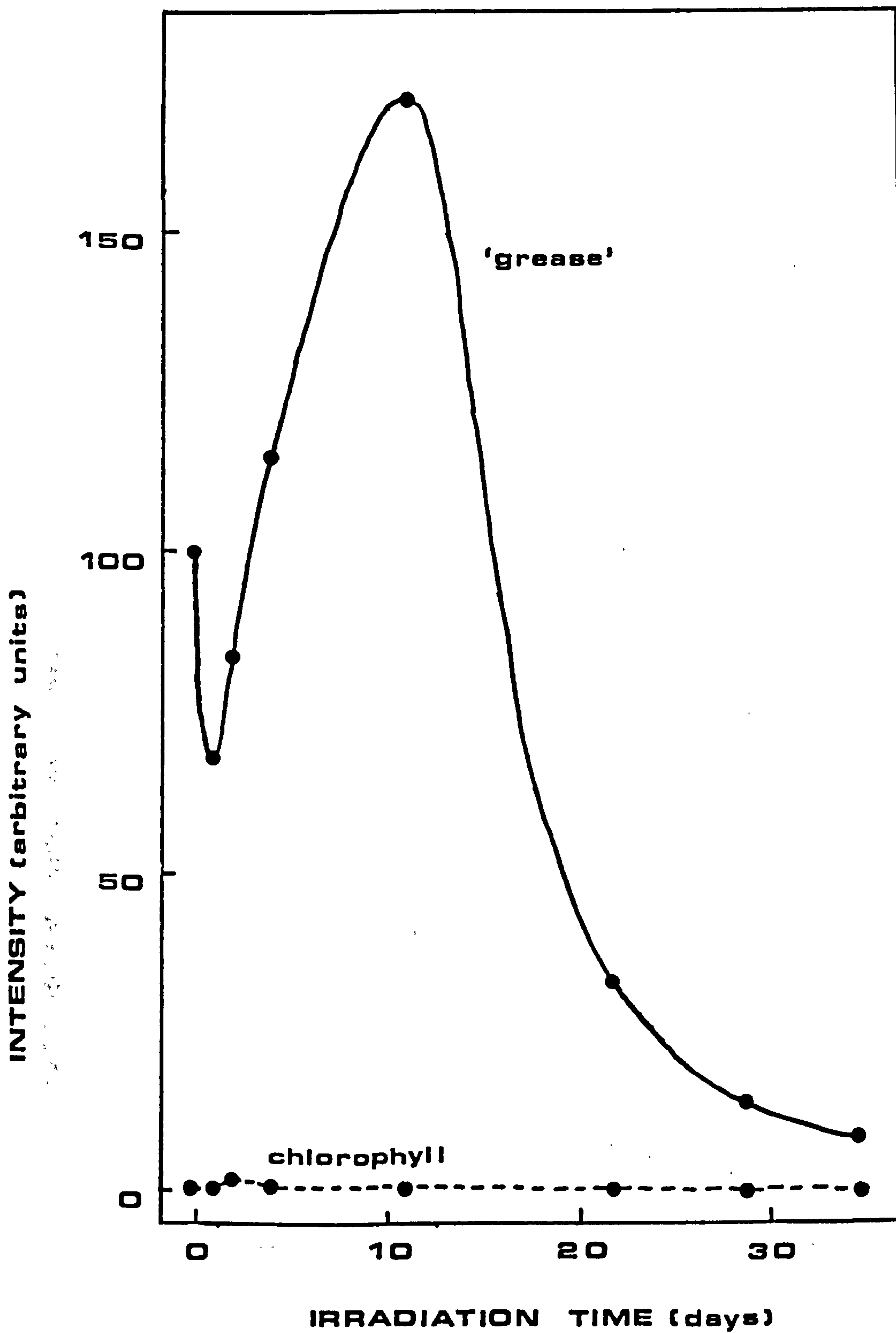


Figure 8.4 Variation in fluorescence intensity of Merino wool grease with time of Blacklight (UV) irradiation (excitation at 350 nm = 'grease', excitation at 400 nm = chlorophyll).

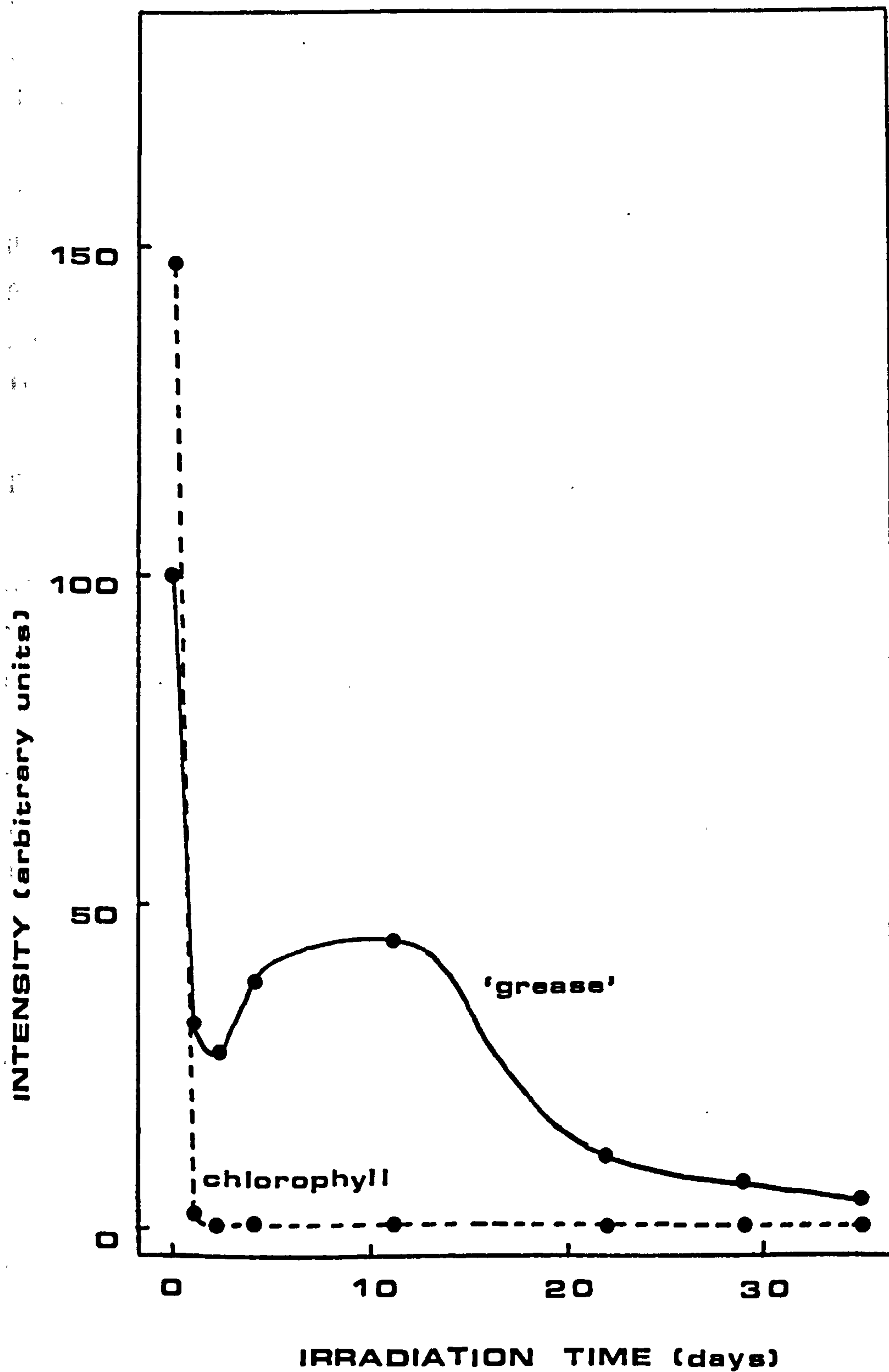


Figure 8.5 Variation in fluorescence intensity of W1 with time of Blacklight (UV) irradiation (excitation at 350 nm = 'grease', excitation at 400 nm = chlorophyll).

The fluorescence of the 'grease' component of Merino wool grease (Figure 8.4) first decreased slightly then increased greatly (to a higher level than that initially observed) before decreasing substantially. The variation in chlorophyll fluorescence is likely to be connected to experimental error as the amount present was so small. The fluorescence of the 'grease' component of W1 (Figure 8.5) showed a similar pattern to that of the Merino wool grease, but the magnitudes of the changes were different. The intensity decreased greatly then increased slightly (but to a lower level than that initially observed) before decreasing further. The chlorophyll fluorescence was very intense initially but very rapidly decreased. This is in accord with the knowledge that chlorophyll is light sensitive [19].

The values of the fluorescence intensity of the 'grease' component of Merino wool grease and W1 are restated in Table 8.6 along with the emission maxima of the samples. Most of the peaks are broad. With the Merino wool grease the fluorescence maximum is initially at approximately 400 nm then quickly increases to approximately 430 nm (at day 1) where it remains for a while before decreasing (after day 11) to approximately 400 nm at the end (at day 35). With W1 the fluorescence maximum is initially at 405 nm then increases (but not so quickly) to 430 nm (at day 4) before decreasing to approximately 400 nm at the end (at day 35). By comparing the fluorescence intensities and emission maxima it is clear that there is no correlation between the two, for both the Merino wool grease and W1.

It is apparent from these results that complex photochemical reactions are occurring. A number of processes could be occurring either individually or together or following one another, including:

1. the change of a non-fluorescent species to a fluorescent species.
2. the change of a fluorescent species to a non-fluorescent species.
3. the change of one type of fluorescent species to another type of fluorescent species.
4. the change of one type of non-fluorescent species to another type of non-fluorescent species.

Time (days)	Merino Wool Grease ^a		W1 ^a	
	Fluorescence Intensity ^b	Emission Maxima (nm)	Fluorescence Intensity ^b	Emission Maxima (nm)
0	100.0	390 - 410	100.0	405
1	67.2	420 - 440	31.6	410 - 420
2	84.2	410 - 450	26.8	410 - 440
4	115.0	420 - 440	37.7	430
11	171.3	410 - 450	44.1	410 - 430
22	33.0	380 - 430	10.7	400 - 440
29	14.5	380 - 430	7.1	380 - 440
35	9.0	380 - 420	3.9	380 - 420

^a excitation at 350 nm.

^b fluorescence measured between 360 and 620 and expressed in arbitrary units (initial value = 100).

Table 8.6 Variation in fluorescence intensity and emission maxima for Merino wool grease and W1 upon Blacklight (UV) irradiation.

Figures 8.4 and 8.5 clearly show that the first two processes are happening and Table 8.6 suggests that the third process is happening as well. However, it may just seem that way as a combination of the first two processes, where the fluorescent species produced has a different fluorescence maximum to the one destroyed, could also produce the same effect. It is not possible to tell from these (fluorescence) results whether or not the fourth process is occurring.

Also apparent is a difference between the Merino wool grease and W1. It is possible that the large amount of chlorophyll present in W1 is catalysing reactions; alternatively the greases may have different compositions.

It was observed that over the 35 days of Blacklight irradiation the Merino wool grease went from being soft and yellowy brown in colour to being hard and pale yellow. Over the same period of time W1 went from being soft and dark green brown to being hard and pale yellow.

The UV/visible absorption spectra of the Merino wool grease over the period of irradiation are shown in Figure 8.6. (Both this figure and the corresponding figure for W1, Figure 8.7, are of the solutions used for the fluorescence measurements. Consequently, they have all been made to have the same optical density (0.10) at 350 nm.) For the 'visible' part of the spectrum (>350 nm) the absorbance decreased for 4 days irradiation, then increased after 11 days (but still less than that initially) and carried on increasing to reach a maximum at 29/35 days (much higher than that initially). For the 'UV' part of the spectrum (<350 nm) the absorbance increased slowly for 11 days irradiation, then underwent large decreases to reach a minimum at 35 days.

Figure 8.7 shows the UV/visible absorption spectra of W1 over the period of irradiation. For the 'visible' part of the spectrum the absorbance from the chlorophyll present (having peaks at 411 and 665 nm) rapidly disappeared after 1 day of irradiation but apart from that the spectra showed little difference for 11 days where upon the absorbance increased to reach a maximum at 29/35 days. For the 'UV' part of the spectrum (<350 nm) the absorbance increased slowly for 11 days irradiation, then underwent large decreases to reach a minimum at 35 days.

There is good general agreement between Merino wool grease and W1 concerning the changes in the UV/visible absorption spectra. By comparing these with the fluorescence results in Table 8.6 some further understanding of the photochemical processes occurring can be gained. For the Merino wool grease: from day 0 to day 1 it is unclear what is happening; from day 1 to day 11 there is a change of non-fluorescing visible-absorbing species to fluorescing UV-absorbing species while after day 11 there is a change of fluorescing UV-absorbing species to non-fluorescing visible-absorbing species. For W1 a similar pattern emerges: from day 0 to day 2 it is unclear what is happening (apart from the destruction of chlorophyll); from day 2 to day 11 there is a change of non-fluorescing visible-absorbing species to fluorescing UV-absorbing species while after day 11 there is a change of fluorescing UV-absorbing species to non-fluorescing visible-absorbing species.

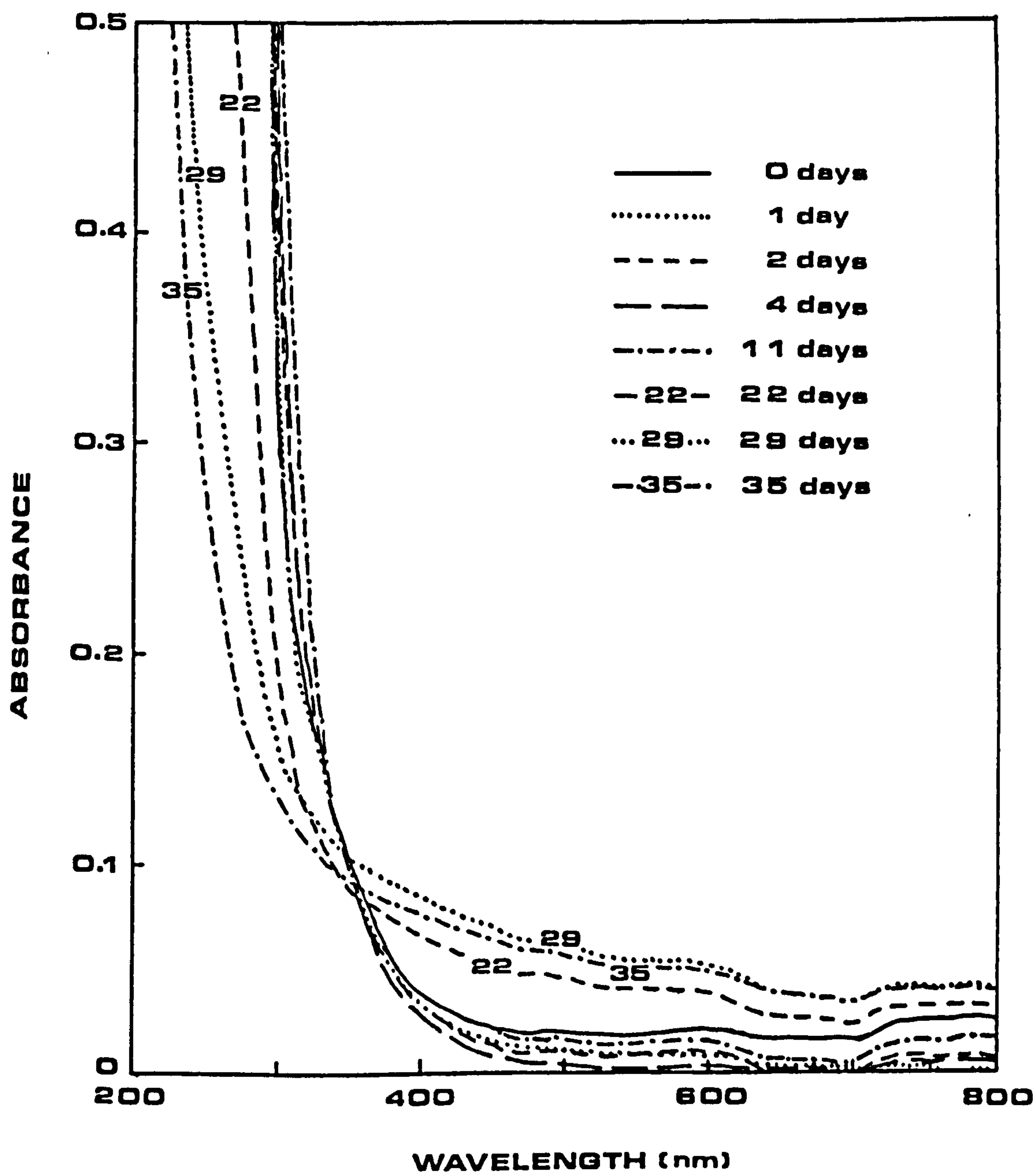


Figure 8.6 Variation in UV/visible absorbance of Merino wool grease with time of Blacklight (UV) irradiation (each sample made to give an optical density of 0.10 at 350 nm).

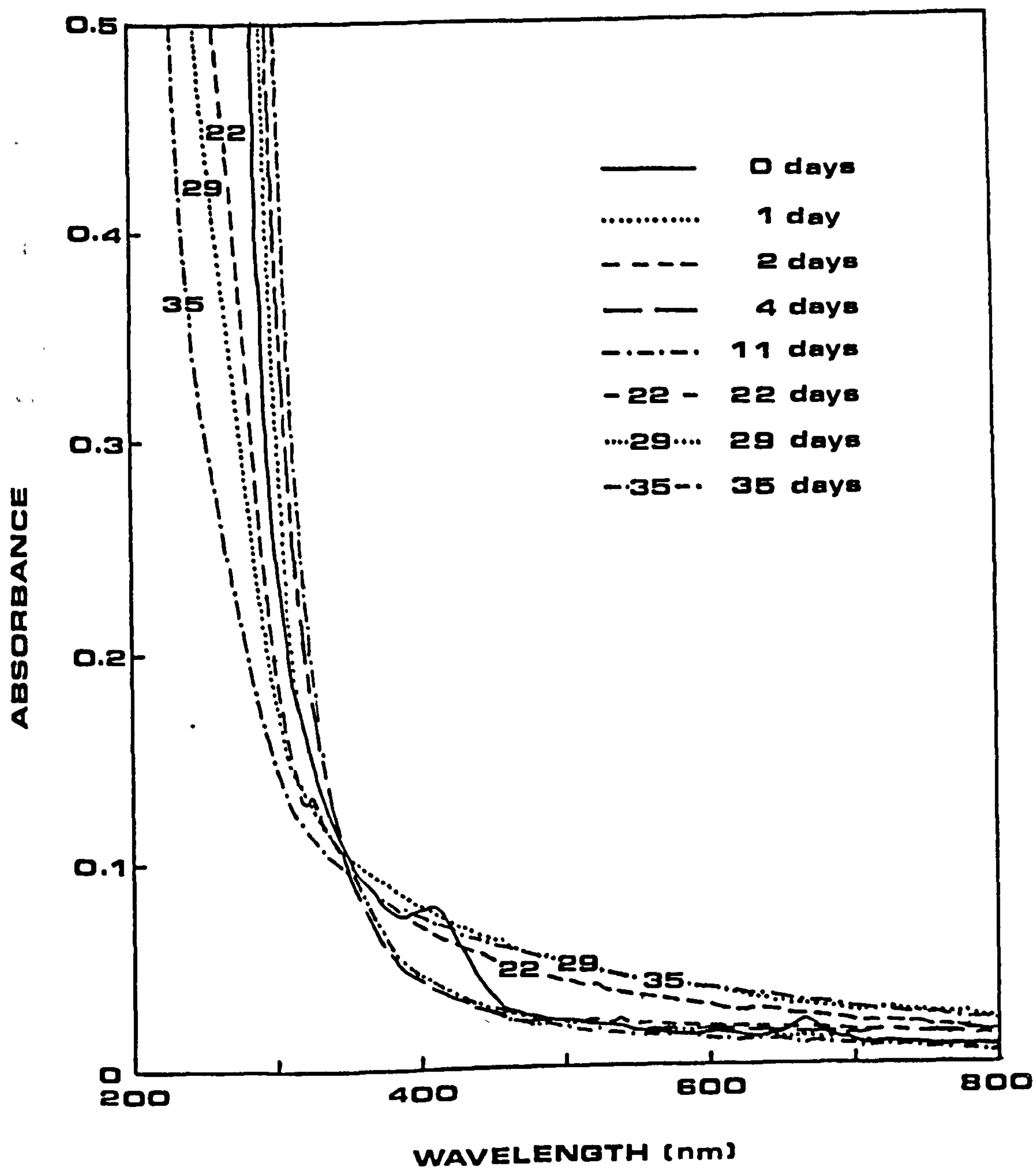


Figure 8.7 Variation in UV/visible absorbance of W1 with time of Blacklight (UV) irradiation (each sample made to give an optical density of 0.10 at 350 nm).

Anderson and Wood examined wool grease recovered by the centrifugation of wool scour liquors [20], and compared unrecovered/recovered wool grease with tip/base waxes obtained by double-shearing a selected sheep. They found that the unrecovered grease correlated with tip wax, exhibiting an increase in UV absorbance in the region 220-280 nm (using 0.03 per cent w/v grease/wax solution in cyclohexane) which is characteristic of auto-oxidised wool wax components [20]. The 'problem' with Anderson and Wood's results is that they only provide a 'snapshot' of weathering, which is a continuous process. Whilst the results presented in this section have monitored the effect of UV irradiation over time this does not exactly copy the effect of weathering, which includes the combined effects of moisture and all of the sunlight spectrum (visible light as well as UV). Further work could usefully be carried out. The effect of visible (blue) light irradiation on the natural fluorescence of wool grease could be usefully investigated as well as examining the difference in the fluorescence of wool wax from root and tip portions of a fleece. The differences in the fluorescence results between Merino wool grease and W1 are interesting and unaccounted for. This could be due to the presence of impurities or simply to a different 'grease' composition. The effect of doping Merino wool grease with e.g. chlorophyll or other wax impurities such as manganese (Section 3.2.1.5) and then irradiating with UV light could also be carried out.

To quickly summarise, UV irradiation of wool grease leads to complex photochemical reactions which ultimately lead to the destruction of fluorescing UV-absorbing species and the generation of non-fluorescing visible-absorbing species.

8.3.4.2 Effect of *In Situ* Irradiation

The effect of *in situ* irradiations using a microscope was examined. No embedding medium was used. Consequently, the problem of the embedding medium influencing the results which was experienced when wool was irradiated *in situ* (Section 6.3.5) could not occur. Three investigations were carried out, using: W1 and UV light, W1 and visible light as well as Croda wool grease and 488 nm laser light.

For the first investigation W1 (already extensively studied in Sections 8.3.2 and 8.3.4.1) was irradiated *in situ* with UV light (broad band 365 nm) and fluorescence spectra recorded at various time intervals (Figure 8.8). (Both Figure 8.8 and Figure 8.9 have not been corrected for the background fluorescence (not shown) arising from the optics etc., which represents most of the non-chlorophyll part of the spectra at $t=8$ minutes.) Fluorescence from both the 'grease' and chlorophyll components (c.f. Figure 8.3) can be seen and both are observed to decrease rapidly upon UV irradiation.

In the second investigation W1 was irradiated *in situ* with visible light (450-490 nm) and fluorescence spectra recorded at various time intervals (Figure 8.9). Again, fluorescence from both the 'grease' and chlorophyll components (c.f. Figure 8.3) can be seen and both are observed to decrease upon visible irradiation, although the rate is noticeably slower than when using UV light (Figure 8.8).

In the third investigation Croda wool grease was irradiated *in situ* with 488 nm (blue) laser irradiation with fluorescence spectra being recorded before and after (Figure 8.10). (The spectra have not been corrected for the small amount of background fluorescence present (35 arbitrary units).) The initial spectrum shows fluorescence from 'grease' and chlorophyll components (c.f. Figure 8.3). Although the amount of chlorophyll fluorescence is small it does nevertheless demonstrate the widespread presence of chlorophyll in wool greases. Upon irradiation the fluorescence from both the 'grease' and chlorophyll components is diminished, particularly the 'grease' component.

However, there is a problem with all the *in situ* results presented here in that they are only 'snapshots' and so might unduly simplify a complex situation (Section 8.3.4.1). Due to the higher light intensities involved different processes may be occurring than those which happen in nature (and in non *in situ* irradiations such as was used in Section 8.3.4.1).

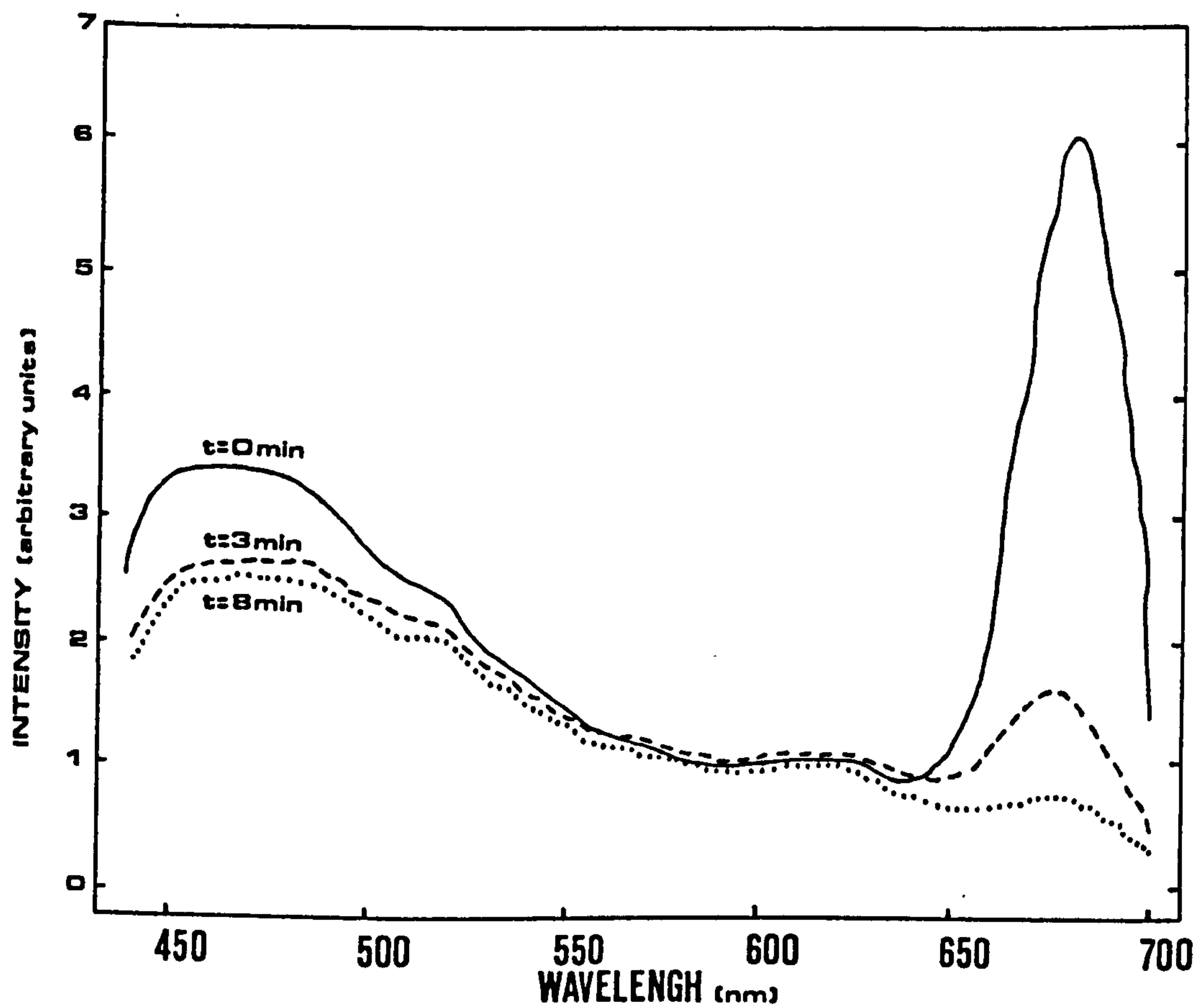


Figure 8.8 Variation in fluorescence spectra of W1 during *in situ* irradiation with UV light (UV excitation).

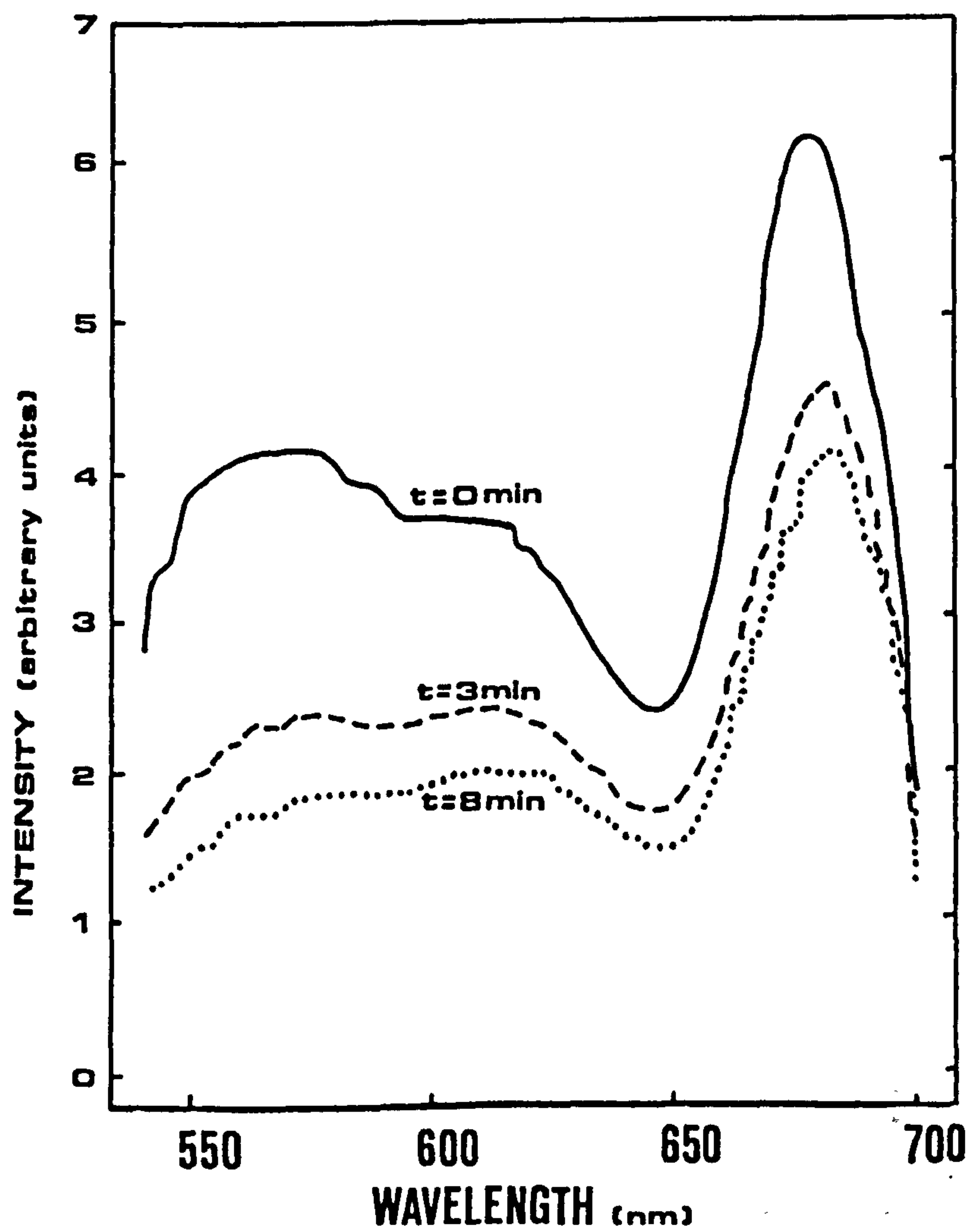


Figure 8.9 Variation in fluorescence spectra of W1 during *in situ* irradiation with visible light (visible excitation).

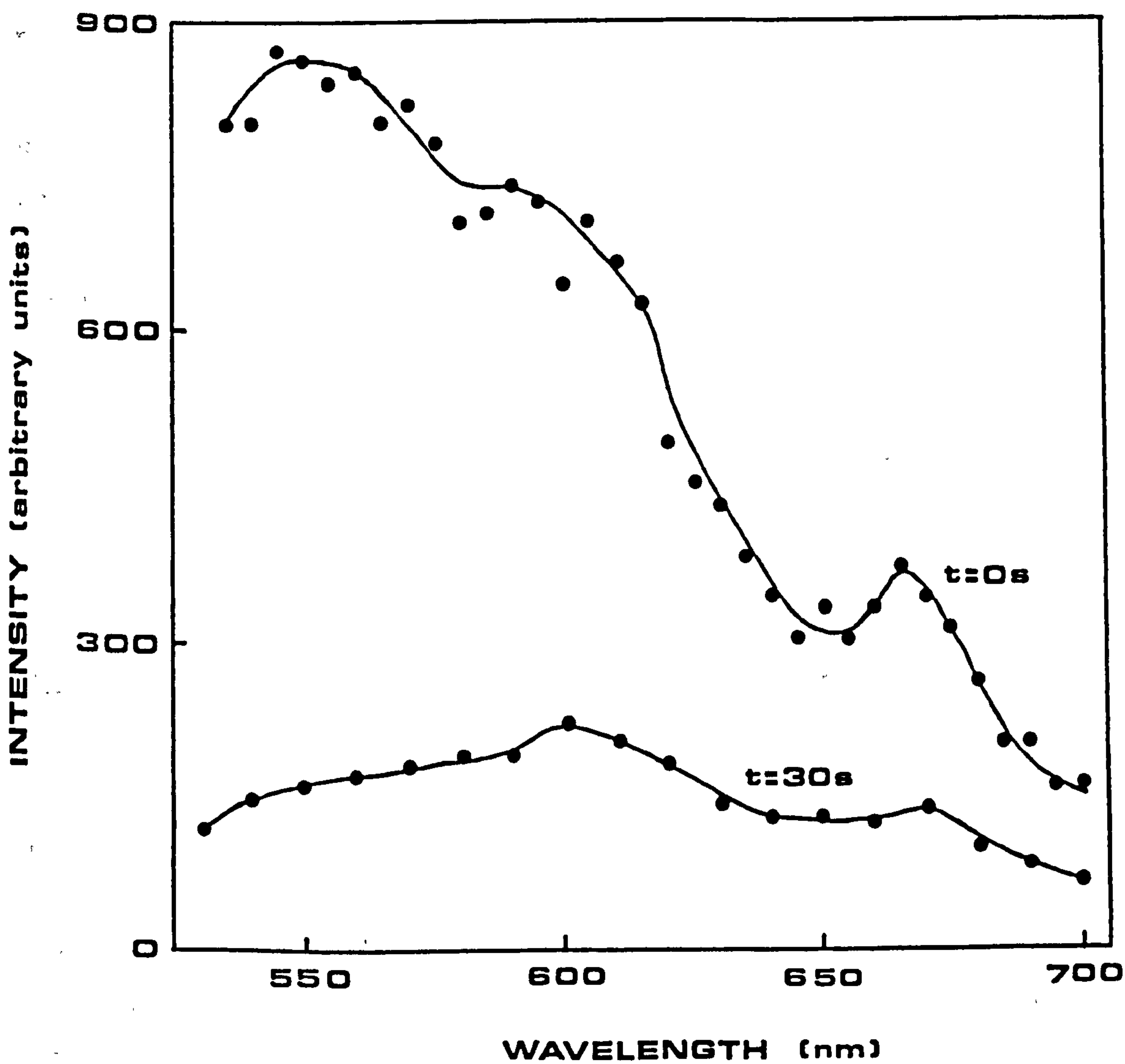


Figure 8.10 Fluorescence spectra of Croda wool grease before and after 30 seconds *in situ* 488 nm laser irradiation (488 nm excitation).

For W1 and UV light (Figure 8.8) the 'grease' and chlorophyll components decrease approximately at proportionally the same rate unlike Blacklight (UV) irradiation (Figure 8.5) where the chlorophyll component decreased much faster. In comparison, with W1 and visible light (Figure 8.9) the 'grease' component decreases quicker than the chlorophyll component (although both do so at a slower rate than with UV light). This is reiterated by the Croda wool grease with 488 nm laser irradiation where the proportional decrease of the 'grease' component is more than of the chlorophyll component. However care has to be taken here as it was observed with blue light irradiation of wool (Section 6.3.4) that intense laser irradiation (442 nm or 488 nm) could cause different effects than those observed with blue (Northlight) irradiation.

The only previous report of the effect of light on the fluorescence of wool wax was by van Raalte [4]. He left some fuller's earth refined sheep fat, which exhibited luminescence, in a test tube in sunlight and after several weeks did not observe any luminescence. Since the test tube was probably made out of glass it would have filtered out some of the UV portion of sunlight (c.f. Figure 4.2). Hence, the photodegradation of the sheep fat must have been stimulated by mainly visible irradiation. The present results agree with van Raalte's and show that visible irradiation causes a decrease in the intensity of the natural fluorescence of wool grease.

It is interesting to compare the effect of light irradiation on wool and wool grease. For wool, UV irradiation causes an increase in fluorescence intensity while blue light (not laser) causes no change. However, for wool grease, both UV and blue light irradiation cause a decrease. Clearly the species' responsible for the fluorescence of both wool and wool grease cannot be identical, although there may be constituents common to both.

These *in situ* irradiation studies of wool grease confirm the destructive effect of UV light on both the 'grease' and chlorophyll components of wool grease and reveal that blue light causes similar effects (although then the 'grease' component is more affected than the chlorophyll component).

8.4 CONCLUSIONS

Wool yolk is highly fluorescent. The amount of wool yolk/intensity of wool yolk fluorescence varies along a Merino wool fibre from 'zero' at the tips to intense at the roots. This is directly opposite to the fluorescence of the Merino fibre which, agreeing with previous results, varies from 'intense' fluorescence at the tips to 'zero' fluorescence at the roots.

The fluorescence of wool grease consists of two components - 'grease' and chlorophyll. The 'grease' fluorescence varies in intensity and emission maximum upon variation in excitation wavelength and reveals the presence of a number of chromophores/components. An attempt to separate these and so help identify them was only partly successful as each of nine fractions obtained by preparatory tlc exhibited the same variable spectra. There are two types of chlorophyll present - chlorophyll a (emission maximum 672 nm) and chlorophyll b (emission maximum 660 nm). Of the two, chlorophyll a is by far the most prevalent. Chlorophyll could be detected in all the wool greases examined. As well as being easily observable in pigmented New Zealand wool greases it could also be found in low levels in Merino and Croda wool greases.

Weathering was found to result in a decrease in the fluorescence intensity arising from both the 'grease' and chlorophyll components. Artificial (Blacklight) UV irradiation revealed (apart from the rapid destruction of chlorophyll) that complex photochemical reactions were occurring. After an initial decrease in fluorescence intensity (for unknown reasons), there is a change of non-fluorescing visible-absorbing species to fluorescing UV-absorbing species before a final change in the latter to the former. The relative size of the changes was different for the two different wool greases examined, one of which was a heavily pigmented New Zealand wool grease. This suggests further work looking at the effect of impurities, especially chlorophyll, being present during UV irradiation.

In situ studies using blue light suggest that it too causes a (slower) decrease in fluorescence intensity - but proportionally faster for the 'grease' component than for the chlorophyll component.

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CHAPTER 9

THE ROLE OF WOOL YOLK

IN THE PHOTOYELLOWING OF WOOL

9.1 INTRODUCTION

In the previous chapter, Chapter 8, it was observed (Figure 8.1) that on a Merino fibre the essentially non-fluorescent wool is covered in very fluorescent wool yolk (the relevant nomenclature is described in Section 3.1). This raises the issue of the relationship between the wool and wool yolk and the possible interactions that may occur. There are a number of possibilities:

1. 'All the fluorescent species of the wool arise through diffusion of such species from the wool yolk'. Although both wool and wool grease show a similar variable fluorescence emission maximum on varying the wavelength of excitation, the similarity stops there. Wool grease shows a decrease in fluorescence intensity upon an increase in excitation wavelength over the range 300-400 nm whereas an opposite trend is observed for wool (compare Figures 8.2 and 8.3 with Figures 2.13 and 2.14 as well as [1]). Additionally, the two behave differently upon exposure to light as described in Section 8.3.4.2. 'For wool, UV irradiation causes an increase in fluorescence intensity while blue light (not laser) causes no change. However, for wool grease, both UV and blue light irradiation cause a decrease. Clearly the species' responsible for the fluorescence of both wool and wool grease cannot be identical, although there may be constituents common to both'.
2. 'Wool yolk promotes photoyellowing'. The opposite of this, namely whether wool yolk protects the fibre, was discussed at length in Section 3.2.3.2 in one of the introductory chapters. There it was concluded that suint has a protective effect against photoyellowing but the effect of the natural level of wax was unclear. In Chapter 6 it was observed that wool yolk exhibited a definite protective effect on the wool fibre upon UV irradiation (Figure 6.1), but no apparent protective effect (or any other effect) upon blue light irradiation (Figures 6.2-6.4). Consequently, the wool yolk rather than promoting photoyellowing, acts to protect the wool fibre!

3. 'Some (fluorescent) substances have migrated from the wool yolk into the wool fibre'. That it is possible for substances to diffuse into a fibre is well known as it is the basis of the dyeing of wool (Section 2.6.1). The questions then arise as to whether substances do regularly or can possibly migrate into wool in nature. Comparing the composition of wool wax (Section 3.2) with the composition of the internal lipids (Section 2.6.3) indicates that the two are separate entities. This shows that the internal lipids did not originate in the wool wax [2-6] and hence, that diffusion into the fibre does not normally occur on a large scale in nature. This does not rule out the possibility of migration happening. Chipalkatti *et al.* [7], supported by Kenkare *et al.* [8,9], have shown that (unidentified) pigments present in the suint are responsible for canary coloration in Indian raw wools. It was concluded that the pigments enter the fibre when the grease content is low. (This indicates that the grease protects the fibres from the suint, which is in accord with the knowledge that the suint is deposited over the wax in the wool follicle (Section 3.1).) Although this shows that migration can occur in nature under certain special circumstances it still does not answer the question as to whether migration can occur (on a small scale) under normal conditions in nature.
4. 'Migrated substances sensitise photoyellowing'. This presupposes that migration can occur and is a variation on the third possibility outlined above. The question arises as to what do the substances do once they have migrated. The case of the canary coloration of Indian raw wools illustrates the possibility of migrated substances being coloured (and so making the fibre coloured). Another possibility is that migrated substances may sensitise photoyellowing. Given that the second possibility (wool yolk promotes photoyellowing) was found to be false, the substances would have to migrate, lay dormant until after the fibre had been cleaned and then 'spring into action' as sensitisers!

This chapter describes experiments which have been carried out in order to investigate the latter two possibilities i.e. to see, firstly, if migration occurs under natural conditions, and secondly, if (artificially) migrated substances sensitise photoyellowing (as measured by an increase in fluorescence intensity).

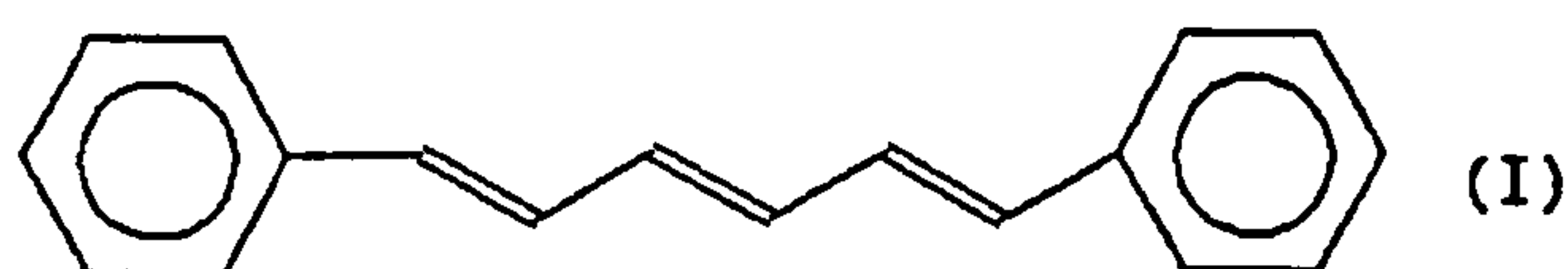
9.2 EXPERIMENTAL

A detailed explanation of the main materials and techniques used can be found in Chapter 4.

9.2.1 Description of Probes

A number of chemicals were used as fluorescent probes and/or sensitisers and are described here. All were used as obtained from the supplier, without further purification.

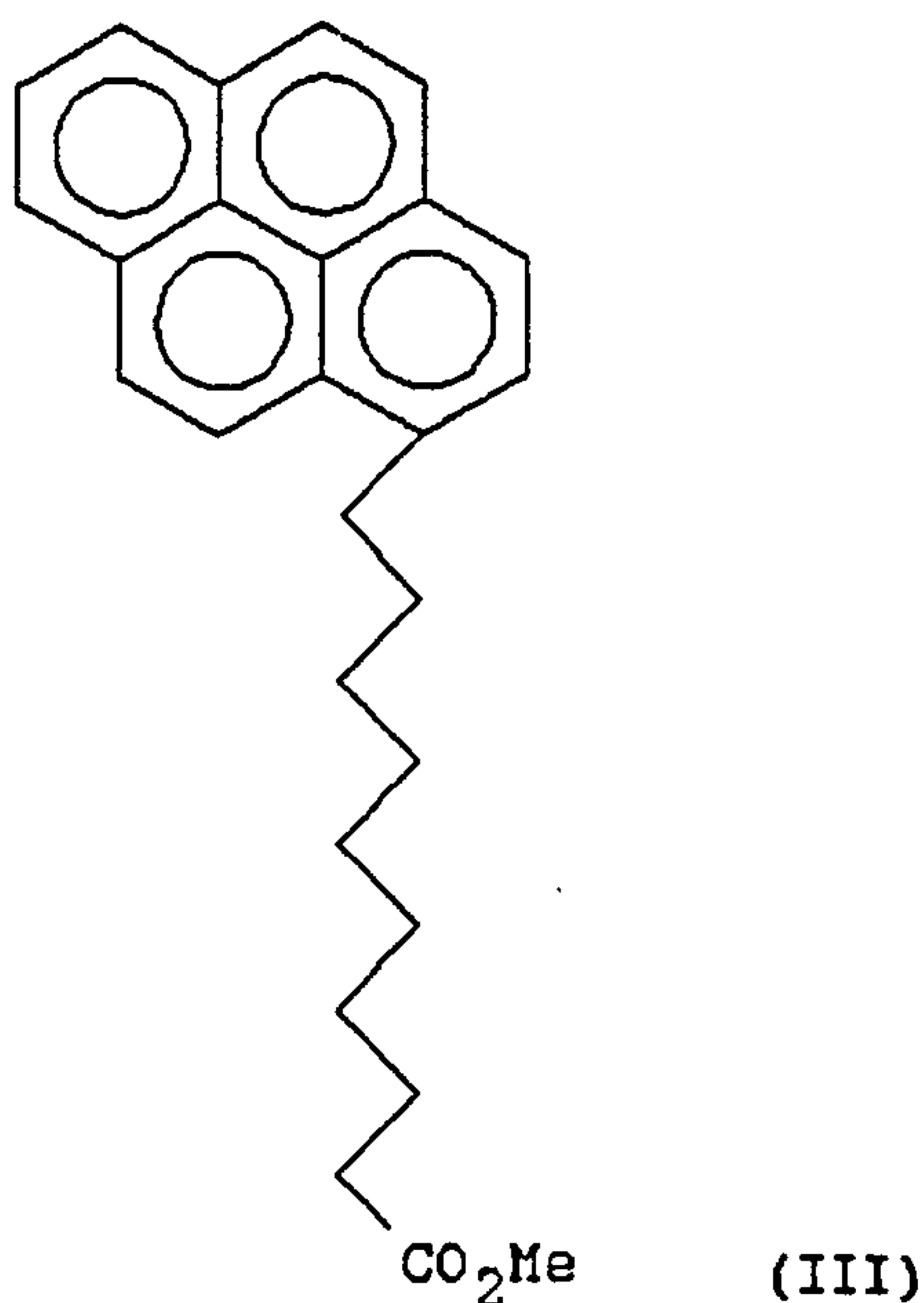
1,6-Diphenyl-1,3,5-hexatriene (I) was obtained from Aldrich. The UV/visible absorption spectrum of I in toluene showed absorption maxima at 342, 359 and 379 nm. The same solution gave fluorescence emission peaks at 406, 428 and 452 nm on excitation at 360 or 380 nm. Examination of the fluorescence emission at 430 nm revealed that it was due to excitation peaks at 360 and 379 nm with a shoulder at 344 nm.



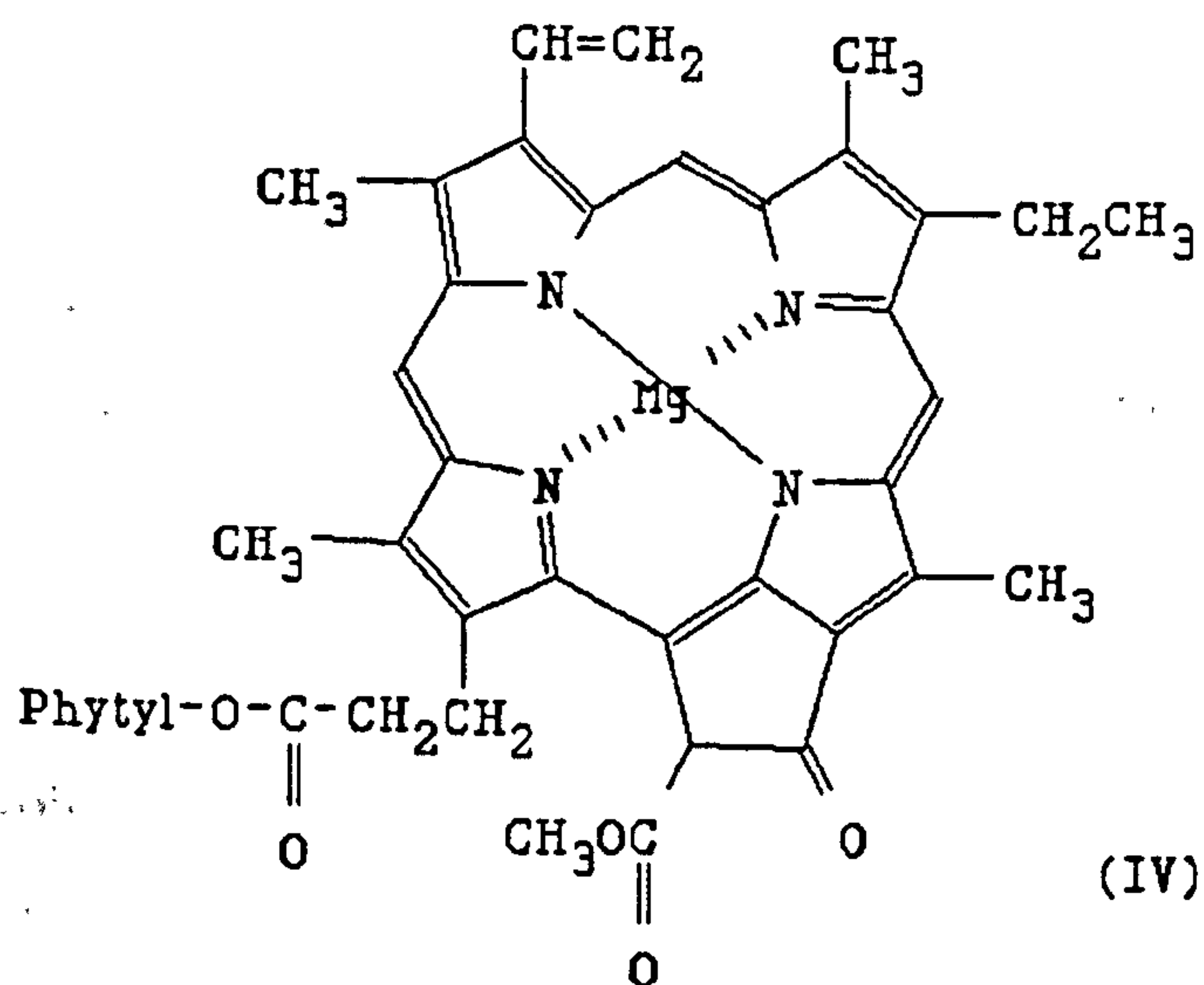
Parinaric acid (II) (also known as β -parinaric acid and 9,11,13,15-octadectetraenoic acid (all trans form)) was obtained from Pharmacia. The UV/visible absorption spectrum of II in cyclohexane showed absorption maxima at 288, 301 and 316 nm, which arise from the tetraene chromophore [10]. The same solution gave a fluorescence emission peak at 404 nm on excitation at 300 nm. Examination of the fluorescence emission at 400 nm revealed that it was due to excitation peaks at 290, 303 and 316 nm.



Methyl 1-pyreneonanoate (III) was obtained from Molecular Probes. The UV absorption spectrum of III in cyclohexane showed maxima at 244, 266, 277, 327 and 343 nm. The same solution gave fluorescence emission peaks at 376, 395 and 414 nm on excitation at 243, 276, 326 or 343 nm. (In addition an emission peak at 282 nm arose from excitation at 243 nm.) Examination of the fluorescence emission at 375 and 395 nm revealed that they were due to excitation peaks at 244, 266, 276, 313, 326 and 343 nm.



Chlorophyll a (IV) (from *Anacystis nidulans* algae; free of chlorophyll b) was obtained from Sigma Chemical Company Ltd. The UV/visible absorption spectrum of IV in toluene showed maxima at 411, 430 and 665 nm. The same solution gave a fluorescence emission peak at 670 nm with a shoulder at approximately 720 nm upon excitation at 430 or 665 nm. Examination of the fluorescence emission at 670 nm revealed that it was due to excitation peaks at 413, 432, 620 and 667 nm.

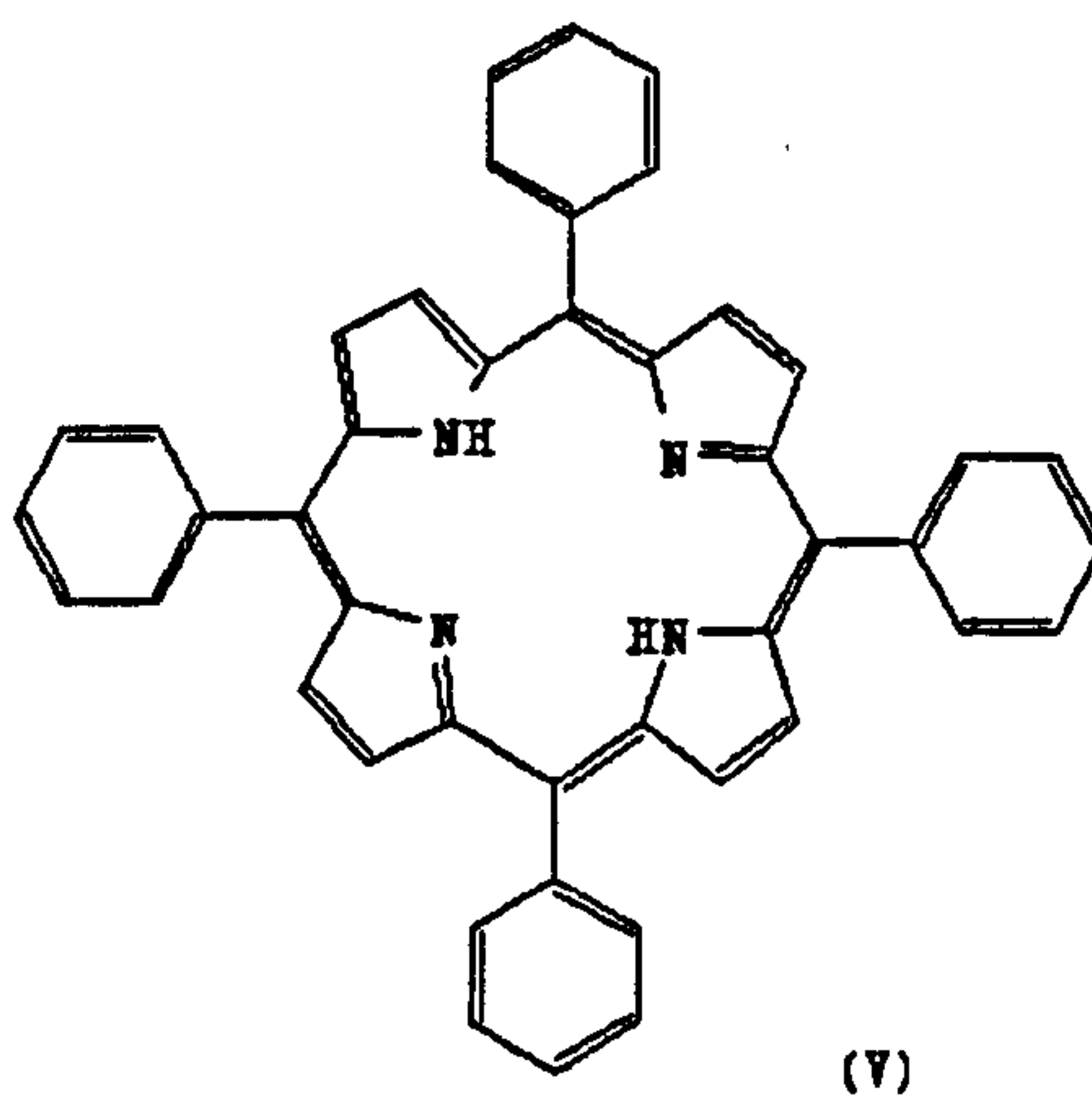


where Phytyl is



(Replacement of the methyl group (CH_3) adjacent to the ethyl group (CH_2CH_3) by an aldehyde group (CHO) would yield chlorophyll b [11].)

meso-Tetraphenylporphyrin (V) was obtained from Mr G. Bashirians, City University. It has been reported that V has UV/visible absorption maxima at approximately 420 and 515 nm in benzene [12].



9.2.2 Migration

The underlying strategy entailed applying a fluorescent probe to the yolk/grease on the outside of fibres in staples, leaving the staples for a period of time and then examining the fibres by fluorescence microscopy to see if any of the probe had migrated into the fibres.

One preliminary and two main approaches to the application of the fluorescent probe were adopted. The toluene (Rathburn, glass distilled grade) was dried by distilling it over sodium before use. Wool from Merino fleece 2 was used.

9.2.2.1 Preliminary Approach

The 'first step' involved dipping a staple of clean wool into (warm) liquid Merino wool grease. However, this caused the weight of the staple to increase by approximately 2500%. As this bore no relation to natural conditions this approach was pursued no further (c.f. Table 3.1).

9.2.2.2 First Approach

This initially involved dipping dry clean wool staples into a solution of wool grease in toluene and was used for the application of 1,6-diphenyl-1,3,5-hexatriene (I) and parinaric acid (II). Wool was very carefully cleaned using Cleaning Method 2.

I: Merino wool grease (4.0g) and I (70mg) were dissolved in toluene (100 ml). Wool (1.0g) (which had been dried by leaving under vacuum over phosphorus pentoxide) was dipped into the grease solution for 30 seconds before being removed. The wool was dried by initially being squeezed with tweezers, before being damped with filter paper and then left to dry overnight under a stream of nitrogen. The weight of the wool had only increased by approximately 20% (to 1.2g). The wool was dried (by leaving under vacuum over phosphorus pentoxide) and the grease solution concentrated to 40 ml. The wool was then dipped again into the solution and left to dry in the dark. The weight of the wool had increased

by approximately 250% (to 3.6g). Examination of the wool using a 375 nm tlc lamp revealed a yellow fluorescence indicating that I had adhered to the wool. The wool was stored in the dark.

After being left for 3 weeks the wool was sampled. The portion (about one third of the total) was dried by leaving under vacuum over phosphorus pentoxide, before being cleaned (2 x 25 ml toluene, ultrasound, approximately 2 minutes) and left to air dry in the dark. An attempt to prepare cross sections of fibres (Section 4.4.2.1) failed as the cellulose acetate used to embed the fibres apparently dissolved I causing it to spread out over the entire bed of fibres and to the edge of the cellulose acetate film.

After being kept for 27 weeks another portion was sampled. This was cleaned using Cleaning Method 3 except that toluene was used instead of t-butanol.

II: Merino wool grease (3.0g) and II (39 mg) were dissolved in toluene (40 ml). Wool (1.0g) (which had been dried by leaving under vacuum over phosphorus pentoxide) was dipped into the grease solution and left to dry in the dark. The wool was stored in the dark.

After being left for 22 weeks the wool was sampled. This was cleaned using Cleaning Method 3 except that toluene was used instead of t-butanol.

I and II: The 27 week sample of the I treated wool and the 22 week sample of the II treated wool were then treated similarly. Cross sections were separately prepared using a Hardy microtome (c.f. Section 4.4.2.1) but using an aqueous PVA polymer support (made of PVA, average molecular weight 10,000) instead of cellulose acetate. Additionally, glycerol was used for embedding the cross sections on microscope slides with no xylene being used. Cross sections were similarly prepared of untreated wool.

Photomicrographs were obtained using UV and visible excitation modes on a LAB16 fluorescence microscope equipped with a 40/0.75 objective (Section 4.4.2.3). Automatic exposure times were used (Ektachrome Professional 50 ASA (Tungsten) colour transparency film).

9.2.2.3 Second Approach

This initially involved spraying wool staples (dried but not cleaned) with a solution of the probe in toluene and was used for the application of methyl 1-pyrenenonanoate (III) and chlorophyll a (IV).

III: III (9.7 mg) dissolved in toluene (5 ml) was sprayed onto wool staples (1.3 g) (which had been dried by leaving under vacuum over phosphorus pentoxide). Inbetween sprayings the wool staples were dried using a hair dryer. Examination of the wool using a 375 nm tlc lamp revealed that III had adhered to the wool. The wool was stored in the dark.

IV: 9.5 ml of a solution of IV in toluene (0.1 mg.ml^{-1}) was sprayed onto wool staples (0.93 g) (which had been dried by leaving under vacuum over phosphorus pentoxide). Inbetween sprayings the wool staples were dried using a hair dryer. Examination of the wool revealed a green colour indicating that IV had adhered to the wool. The wool was stored in the dark.

First and second examinations: The III treated wool was sampled after 13 weeks and the IV treated wool was sampled after 7 weeks. Cross sections of the uncleaned samples were separately prepared using a Hardy microtome (Section 4.4.2.1).

The III treated wool was sampled after 22 weeks and the IV treated wool was sampled after 16 weeks. Each sample was cleaned separately using Cleaning Method 3. (Examination of the t-butanol solutions obtained after soxhlet extraction using a 375 nm tlc lamp revealed a blue fluorescence arising from III and a red fluorescence arising from IV, indicating that the two probes had not degraded on storage.) Cross sections were separately prepared using a Hardy microtome (Section 4.4.2.1).

Photomicrographs were obtained using visible excitation mode on a IM fluorescence microscope equipped with a 32/0.4 objective (Section 4.4.2.4). Automatic exposure times were used (Ektachrome 100 ASA HC (Daylight) colour transparency film). Some photomicrographs were also obtained using UV and visible excitation modes on a LAB16 fluorescence microscope equipped with a 40/0.75 objective (Section 4.4.2.3). Automatic exposure times were used (Ektachrome Professional 50 ASA (Tungsten) colour transparency film).

Third examination: The III treated wool was sampled (all that remained) after 39 weeks and IV treated wool was sampled (all that remained) after 33 weeks. Each sample was cleaned separately using Cleaning Method 5 (scouring). Additionally, an untreated uncleaned portion of wool was also cleaned the same way. In this case the uncleaned wool weighed 1.20 g and the cleaned 1.00 g. For the III treated wool the uncleaned wool weighed 0.54 g and the cleaned 0.41 g. For the IV treated wool the uncleaned wool weighed 0.41 g and the cleaned 0.32 g. Cross sections were separately prepared using a Hardy microtome (Section 4.4.2.1).

Photomicrographs were obtained using UV and visible excitation modes on a LAB16 fluorescence microscope equipped with a 40/0.75 and a 32/0.4 objective (Section 4.4.2.4). Automatic exposure times were used (Ektachrome Professional 50 ASA (Tungsten) colour transparency film).

9.2.3 Effect of Sensitisers

Wool staples, which had been previously cleaned using Cleaning Method 3 but had since been allowed to return to normal regain, were dipped into 50 ml of a 9:1 (v/v) toluene (AnalaR or distilled AnalaR) : methanol (spectroscopic grade or ordinary laboratory reagent grade), with sensitizer (if used), to guarantee uptake [13-15]. The wool was left for approximately one hour at room temperature with occasional stirring before being damped with filter paper and then being left to dry in the dark overnight. Four samples were prepared: the first used 0.52 g of wool (alone), the second used 0.68 g of wool and 24.6 mg of 1,6-diphenyl-1,3,5-hexatriene (I), the third used 0.61 g of wool and 14.7 mg of parinaric acid (II) and the fourth used 0.54 g of wool and 11.4 mg of *meso*-tetraphenylporphyrin (V).

Each solvent treated wool was divided into two parts. The first part (except for the solvent alone treatment) was left for a while before being cleaned (see below). This was to observe any effect the sensitisers had in the dark. The second part was irradiated for 7 days using Irradiation Method 1 equipped with UV lamps (Thorn, 3 x 20W, 600 mm, peak emission 365 nm). During irradiation the staples were turned over once. After irradiation the staples were cleaned (see below). Additionally, wool which had not received any solvent treatment was similarly irradiated, but with no subsequent cleaning.

Initially an attempt was made to clean one of the samples using soxhlet extraction with a 9:1 (v/v) toluene (AnalaR) : methanol (ordinary laboratory reagent grade) mixture. However, bad bumping was experienced, due to the formation of an azeotropic mixture (consisting of methanol (72.5%) and toluene (27.5%) [16]). Consequently, Cleaning Method 3 was used with a 2:1 (v/v) methanol (ordinary laboratory reagent grade) : toluene (AnalaR) mixture. Additionally, the wool was not dried before cleaning.

Small portions of fibres were embedded on a glass slide using glycerol. The portions came from the middle part of the staple so as to exclude the effect of natural weathering. The fibre portions were examined using a LAB16 fluorescence microscope equipped with a Neofluar 16/0.40 objective (Section 4.4.2.3). UV excitation mode was used and all the fluorescence emission produced was monitored. 15 measurements were made for each sample.

9.3 RESULTS AND DISCUSSION

9.3.1 Migration

The underlying strategy entailed applying a fluorescent probe to the yolk/grease on the outside of fibres in staples, leaving the staples for a period of time and then examining the fibres by fluorescence microscopy to see if any of the probe had migrated into the fibres.

Fluorescent probes (or fluorophores) have recently been reviewed [17-19]. 'Almost all fluorophores of practical use for biochemical investigations, are derivatives of aromatic compounds, usually possessing one to five conjugated rings. Possible exceptions with regard to aromaticity, include some rare-earth chelates and certain polyenes, such as parinaric acid and retinol' [17]. Fluorescent probes used in the modification of 'biomolecules' can be of two kinds [17,18]: 1. Covalent fluorescent probes which form strong chemical bonds with specific atoms on the 'biomolecule', and are usually irreversibly bound. 2. Non-covalent fluorescent probes which form a reversible association with the 'biomolecule' by a combination of hydrophobic, dipole-dipole, and ionic interactions.

Both covalent and non-covalent fluorescent probes have been used in wool research. The covalent probe 4-(aminosulphonyl)-7-fluoro-2,1,3-benzoxadiazole has been used to label thiol groups in partially reduced wool fibres [20]. However, the use of the former is more widespread. These are typically used to investigate the uptake of chemicals, e.g. dyes, from solvent systems into wool fibres. A number of chemicals have been used including: Rhodamine B [21-23], Acid Yellow 7 [21], CI Acid Red 52 [24], modified coumarins and pyrazolines [25], simple nonionic pyrazolines [15], 1,3-bis-(1-pyrene)-propane [26,27] and (very recently) 1,6-diphenyl-1,3,5-hexatriene [27].

Non-covalent probes were chosen in the current work to facilitate their movement from the yolk/grease into the wool fibre. 1,6-Diphenyl-1,3,5-hexatriene (I) and parinaric acid (II) [19] as well as methyl 1-pyrenenonanoate (III) [28] are known non-covalent probes, which were used. In the previous chapter, Chapter 8, it was

shown that chlorophyll, especially chlorophyll a (IV) which is highly fluorescent, is a constituent of wool grease. Accordingly IV was also used as a fluorescent probe, mimicking natural conditions.

The first approach involved dipping dry clean wool staples into a solution of wool grease in toluene and was used for the application of I and II.

Wool treated with I was sampled after 3 weeks and an attempt was made to prepare cross sections. This failed as the normal fibre embedding medium used was unsuitable (see Section 9.2.2.2). Samples of wool treated with I (taken after 27 weeks) and II (taken after 22 weeks) were cleaned and cross sections prepared using a different fibre embedding medium. (Cross sections were prepared, here and later in this section, using middle portions of the fibres so as to exclude the effect of natural weathering.) Cross sections were similarly prepared of cleaned untreated wool. Fluorescence micrographs were produced using both UV and visible excitation modes. Although both excite the natural fluorescence of wool it was thought that only the UV excitation mode was useful for exciting the fluorescence of I and II (c.f. excitation maxima Section 9.2.1). Accordingly, the fluorescence micrographs obtained using UV excitation are reproduced in Figure 9.1 (for untreated wool), Figure 9.2 (for I treated wool) and Figure 9.3 (for II treated wool). (Subsequent inspection of solid samples of I, II and III (with no embedding medium) using fluorescence microscopy revealed that fluorescence was observed with both UV and visible excitation!)

A study of Figure 9.1 shows uniform fluorescence across the fibre cross sections with some edges (cuticle) having a more pronounced fluorescence (similar to Figure 5.1b). Figure 9.2, of I treated wool, is similar. Figure 9.3, of II treated wool, again shows uniform fluorescence across the fibre cross sections but with highly fluorescent edges. Clearly, there are no obvious spots or patterns in the fibres due to migration and localisation of the probes into certain morphological regions e.g. the orthocortex. The increased fluorescence of the cuticle of II treated wool (Figure 9.3) suggests that II may have migrated into the cuticle. Alternatively, II may have had a catalytic effect on the surface of the wool; it could not have acted as a photosensitiser as the wool was kept in the dark.

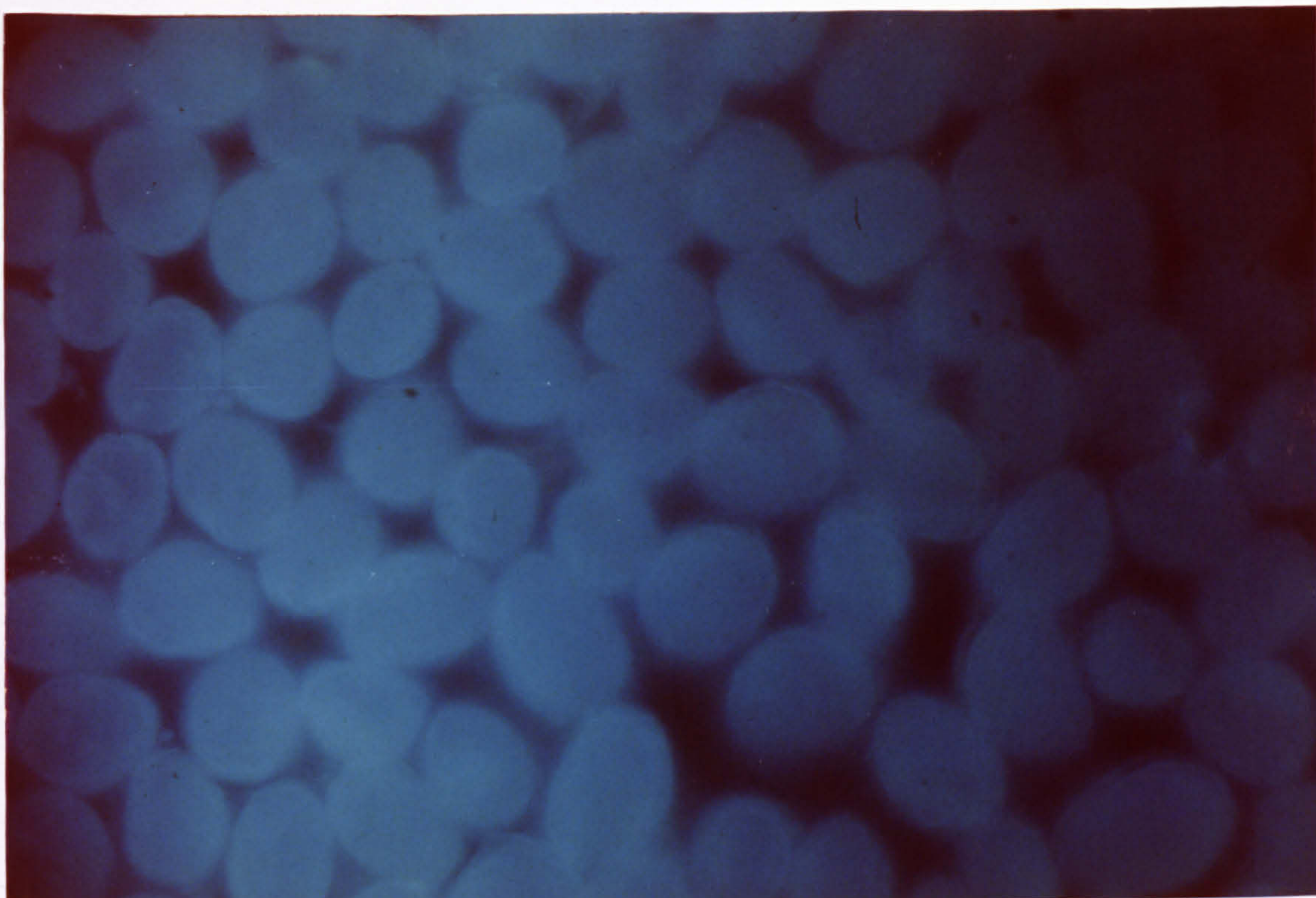


Figure 9.1 Fluorescence micrograph of a cross section of fibres from cleaned untreated wool (UV excitation, magnification x450).

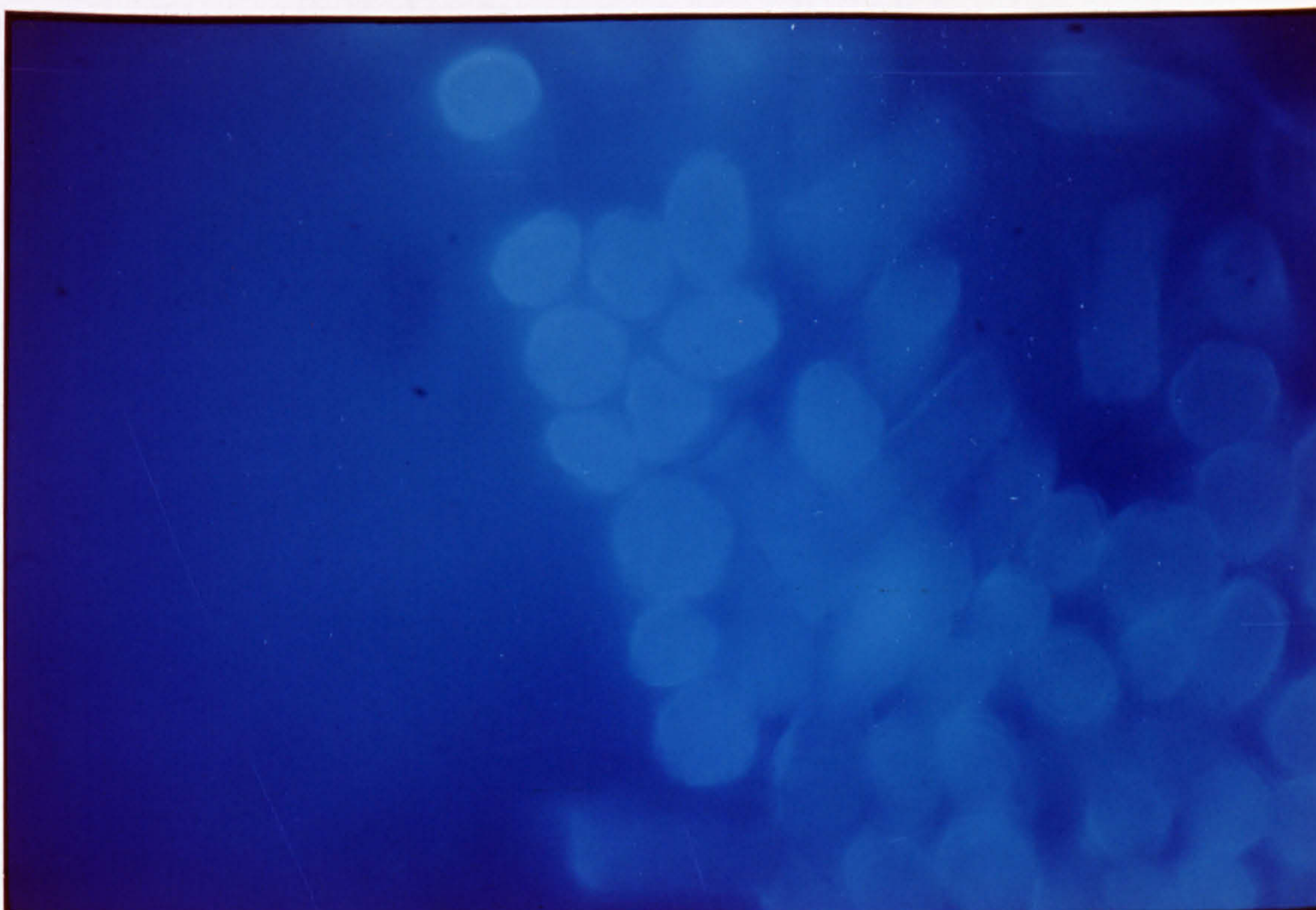


Figure 9.2 Fluorescence micrograph of a cross section of fibres from cleaned wool which had previously been treated with I and left for 27 weeks before being recleaned (UV excitation, magnification x450).

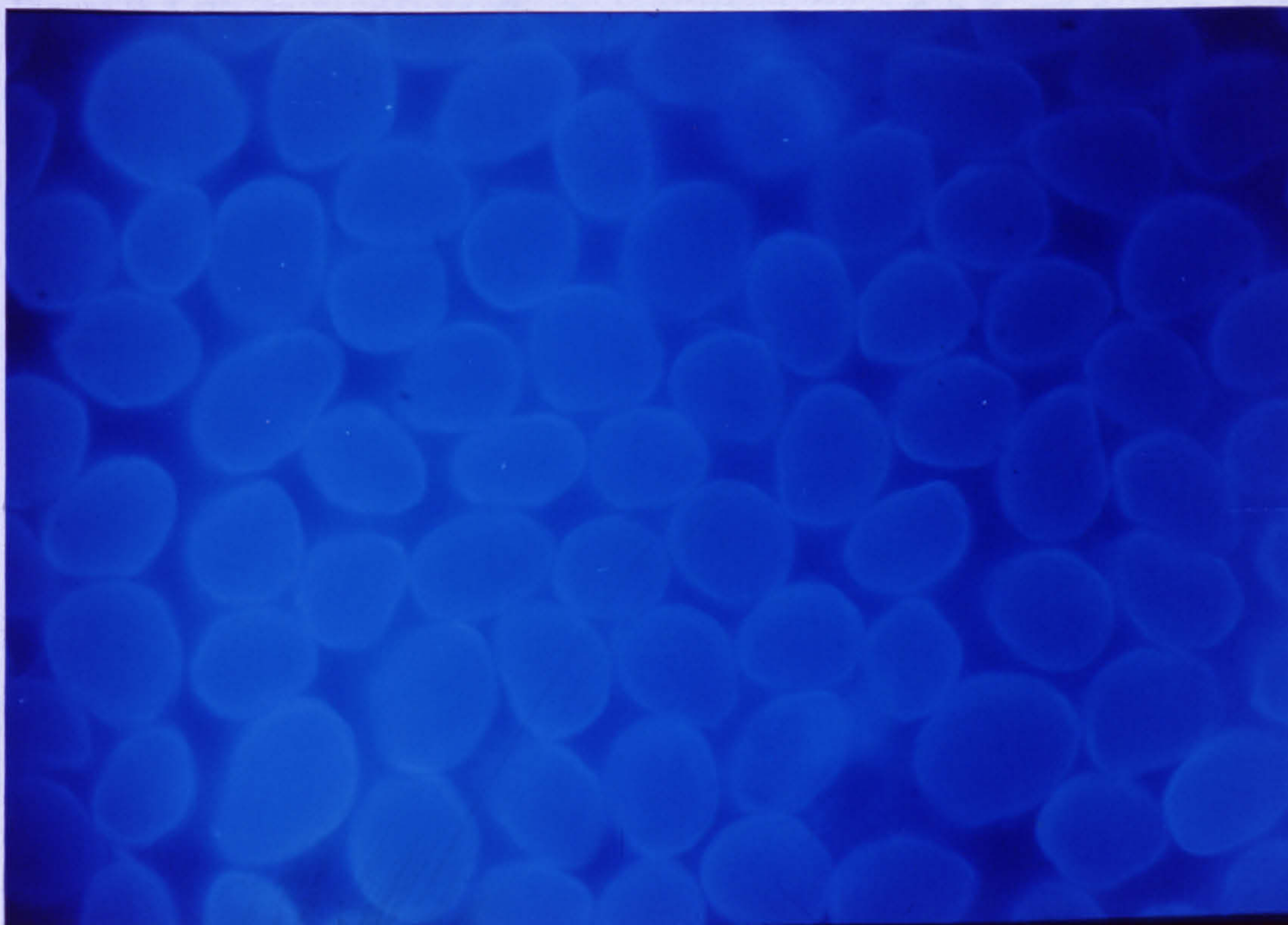


Figure 9.3 Fluorescence micrograph of a cross section of fibres from cleaned wool which had previously been treated with II and left for 22 weeks before being recleaned (UV excitation, magnification x450).

The second approach involved spraying wool staples (dried but not cleaned) with a solution of probe in toluene and was used for the application of III and IV.

Samples of wool treated with III (taken after 13 weeks) and IV (taken after 7 weeks) were obtained (not cleaned) and cross sections prepared. Fluorescence micrographs were obtained.

Fluorescence micrographs of III, using UV excitation, (Figure 9.4) and IV, using visible excitation, (Figure 9.5) are shown.

Figure 9.4 (III treated wool) shows some fibres having edges with a noticeable fluorescence and a spotty interior. Figure 9.5 (IV treated wool) clearly shows the orange/red fluorescence of IV - both surrounding the fibre and in spots across the middle of the fibre. Examination of the fluorescence through the depth of the cross section by moving the cross section in and out of focus (and measuring the fluorescence from IV with the monochromator) revealed that IV was only present on the surface of the cross section. This suggests that IV was spread in the cutting process (c.f. Section 8.3.1) and this was confirmed by observing that in part of Figure 9.5 the inadvertent spreading of the yolk was more obvious. This emphasises that care needs to be taken in sample preparation and consequently led to the next samples being cleaned before examination. The cross section of III treated wool (Figure 9.4) was similarly examined using the confocal focusing of the fluorescence microscope. It was found that the fluorescence was present not only on the surface but also throughout the depth of the cross section. However, as the wavelength of maximum emission (approximately 495 nm upon visible excitation) was similar for the wool and the doped wax it was not possible to tell whether the origin of the spotty fluorescence was from III or the wool itself.

Samples of wool treated with III (taken after 22 weeks) and IV (taken after 16 weeks) were cleaned and cross sections prepared. Fluorescence micrographs of III, using UV excitation, (Figure 9.6) and IV, using visible excitation, (Figure 9.7) are shown.

Figure 9.6 (III treated wool) is similar to Figure 9.4. Figure 9.7 (IV treated wool) shows uniform fluorescence across the fibre cross sections with some fibres having a more pronounced fluorescence. However, it is clear that there is a lack of orange/red fluorescence indicating an absence of IV.

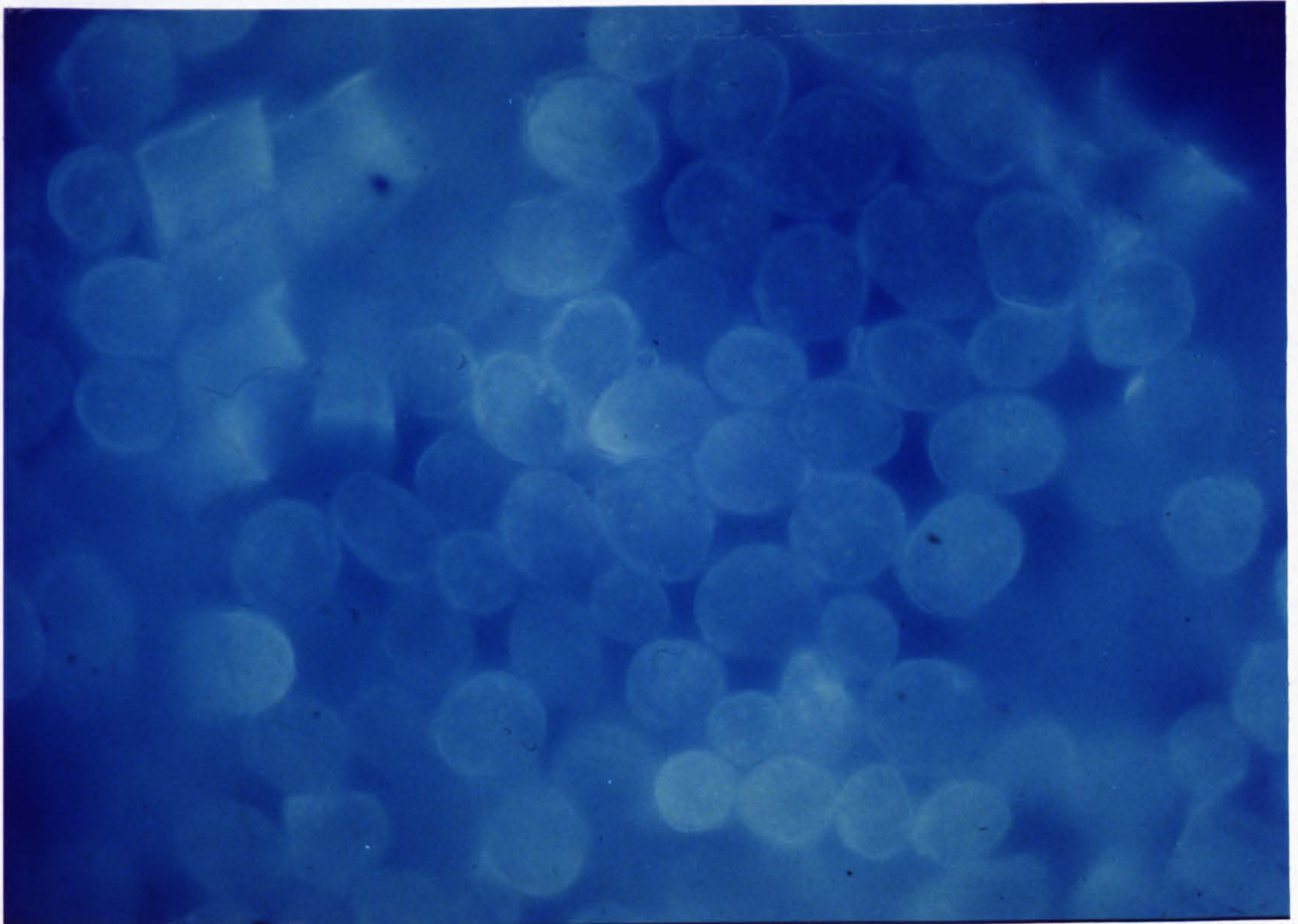


Figure 9.4 Fluorescence micrograph of a cross section of fibres from wool which had been treated with III and left for 13 weeks (UV excitation, magnification x450).

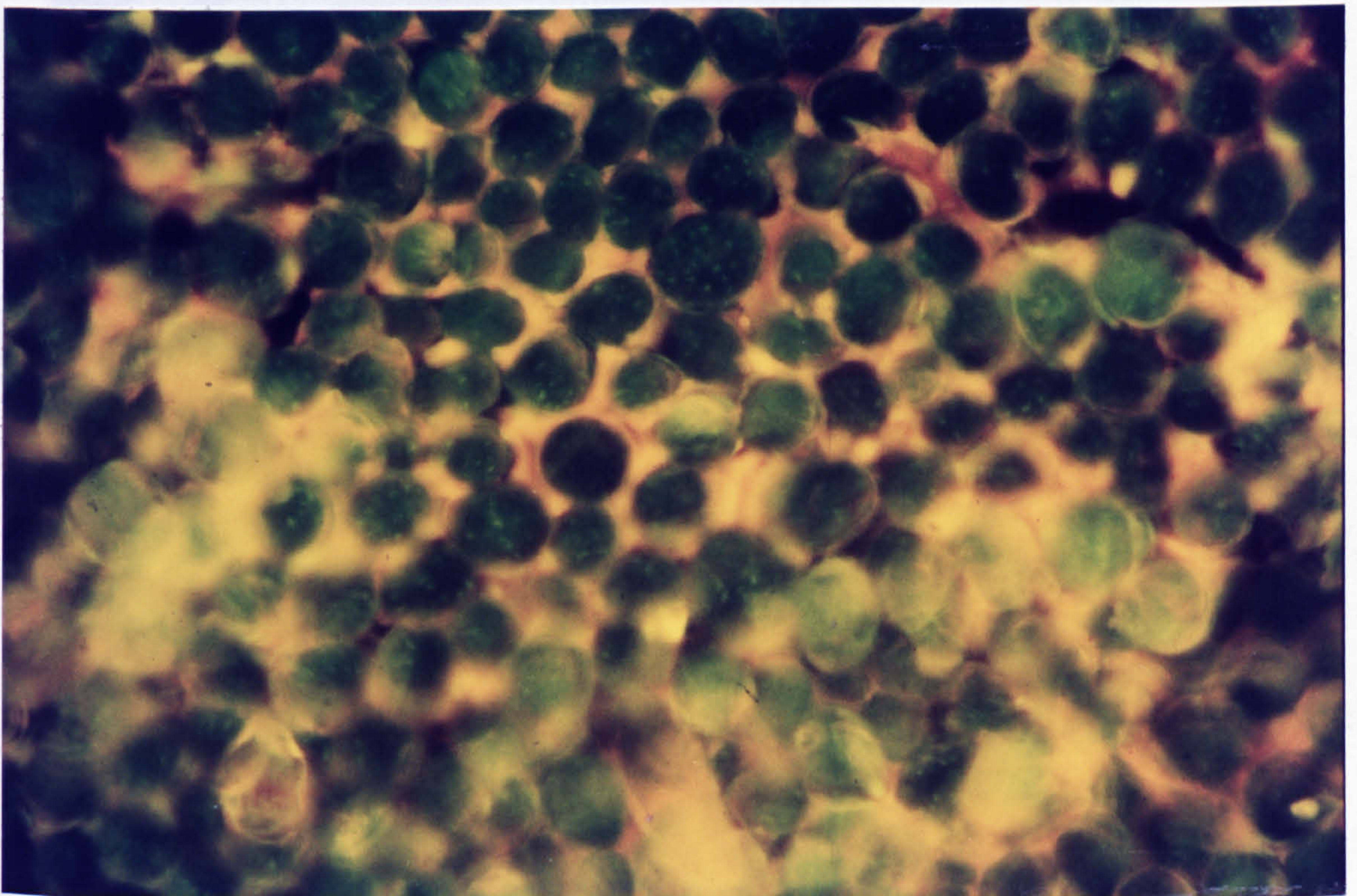


Figure 9.5 Fluorescence micrograph of a cross section of fibres from wool which had been treated with IV and left for 7 weeks (visible excitation, magnification x360).

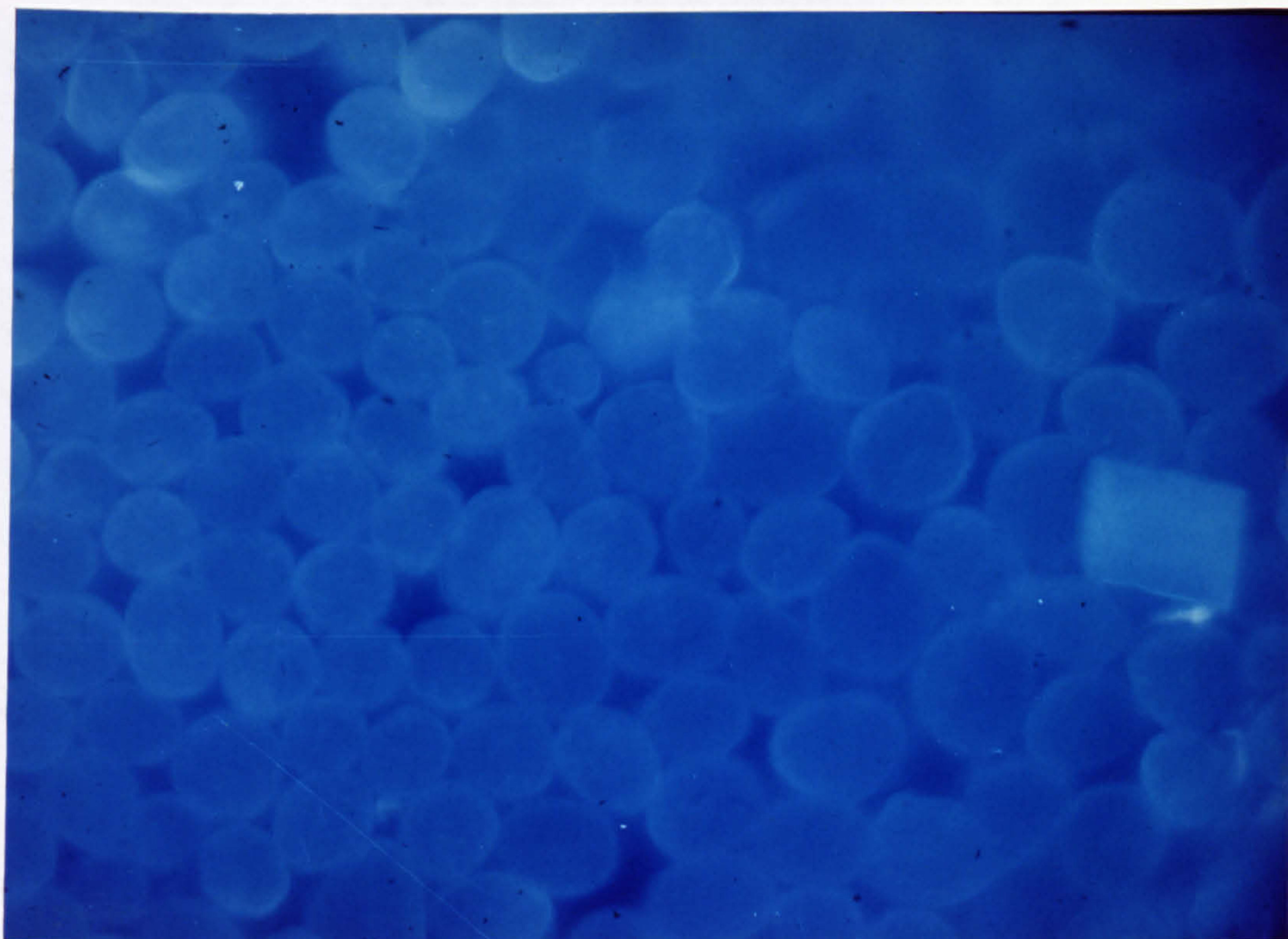


Figure 9.6 Fluorescence micrograph of a cross section of fibres from wool which had been treated with III and left for 22 weeks before being cleaned (UV excitation, magnification x450).

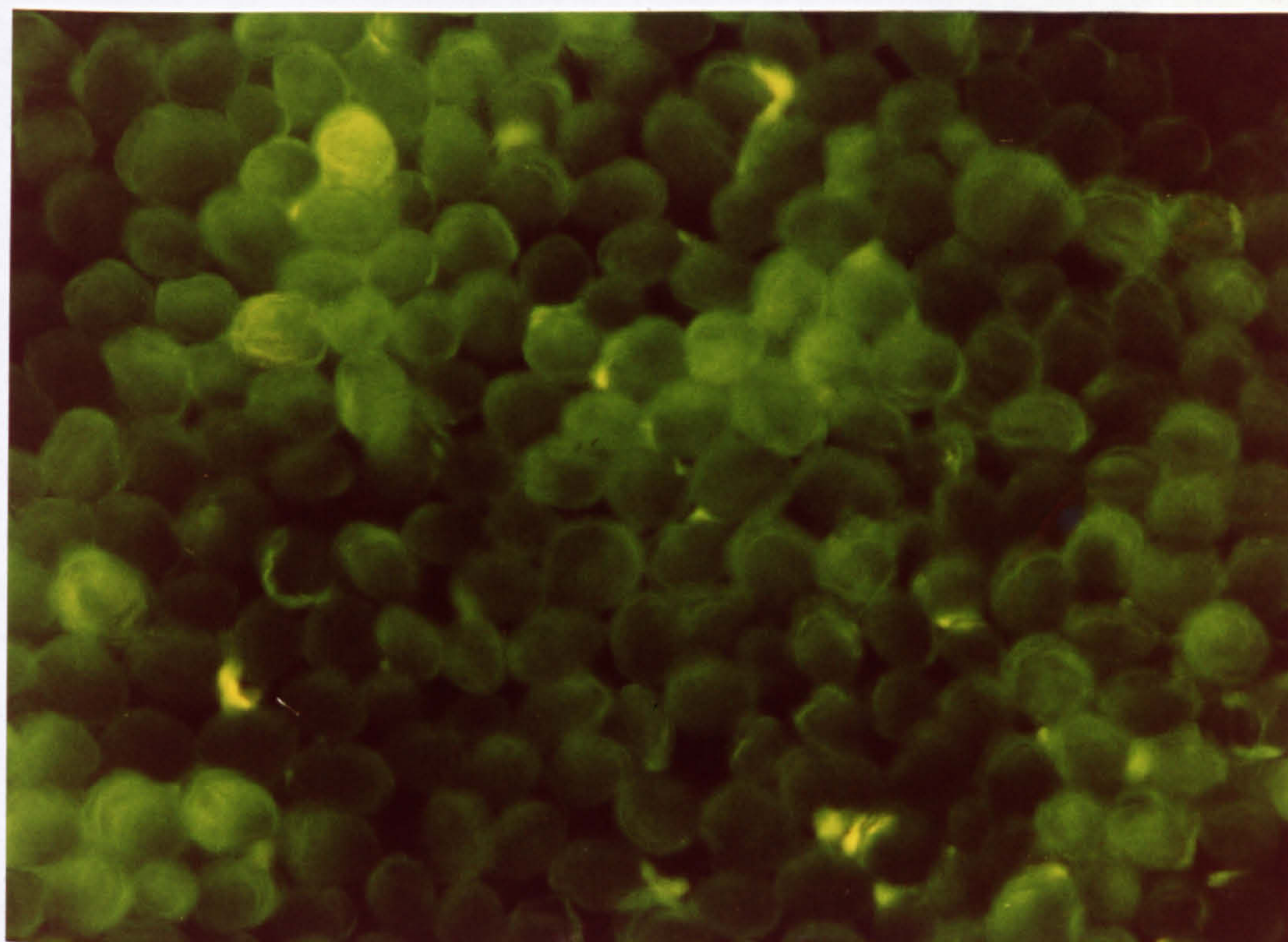


Figure 9.7 Fluorescence micrograph of a cross section of fibres from wool which had been treated with IV and left for 16 weeks before being cleaned (visible excitation, magnification x360).

So far, no fluorescent probe (I-IV) has been unequivocally observed to migrate from the yolk/grease on the outside of the fibres into the fibres. Very special care has been taken to ensure that conditions replicated, as far as possible, natural conditions. The possibility exists that no migration occurs in nature but that some occurs in industrial cleaning. Accordingly, instead of scientific cleaning, samples of wool treated with III (taken after 39 weeks) and IV (taken after 33 weeks) were cleaned using a laboratory scale industrial scouring process. A sample of untreated wool was subjected to the same process. Cross sections were prepared and fluorescence micrographs obtained.

Figure 9.8 (untreated wool, UV excitation) shows fibres having mostly a uniform level of fluorescence with a number having pronounced fluorescence at the edges and one or two being quite intense. Figure 9.9 (III treated wool, UV excitation, partly out of focus) is similar. It is therefore, the fluorescence of the wool which is being observed and not that of III. Figure 9.10 (untreated wool, visible excitation) shows the same as Figure 9.8: fibres having mostly a uniform level of fluorescence with a number having pronounced fluorescence at the edges and one or two being quite intense. Figure 9.11 (IV treated wool, visible excitation) is similar, with no orange/red fluorescence from IV being observable.

Consequently, even after using a laboratory scale industrial scouring process no migration has been observed.

The effect of scouring on the internal lipids of wool has recently been investigated [29]. One of the treatments involved the use of a laboratory simulation of a clean and dirty mill scour. This entailed cleaning 1kg of wool in 50g lots using two bowls each containing 500 ml of water plus a nonylphenoethoxylate detergent. The first and last 50g lots of wool (after rinsing in three changes of water) were regarded as corresponding to wools scoured in clean and dirty liquors. Examination of the lipid extract of both wools revealed the presence of a contaminant in the wool from the dirty bath which was not present in wool from the clean bath. This led to the conclusion that contaminants in the scour bath diffuse into the fibre during the scouring process.

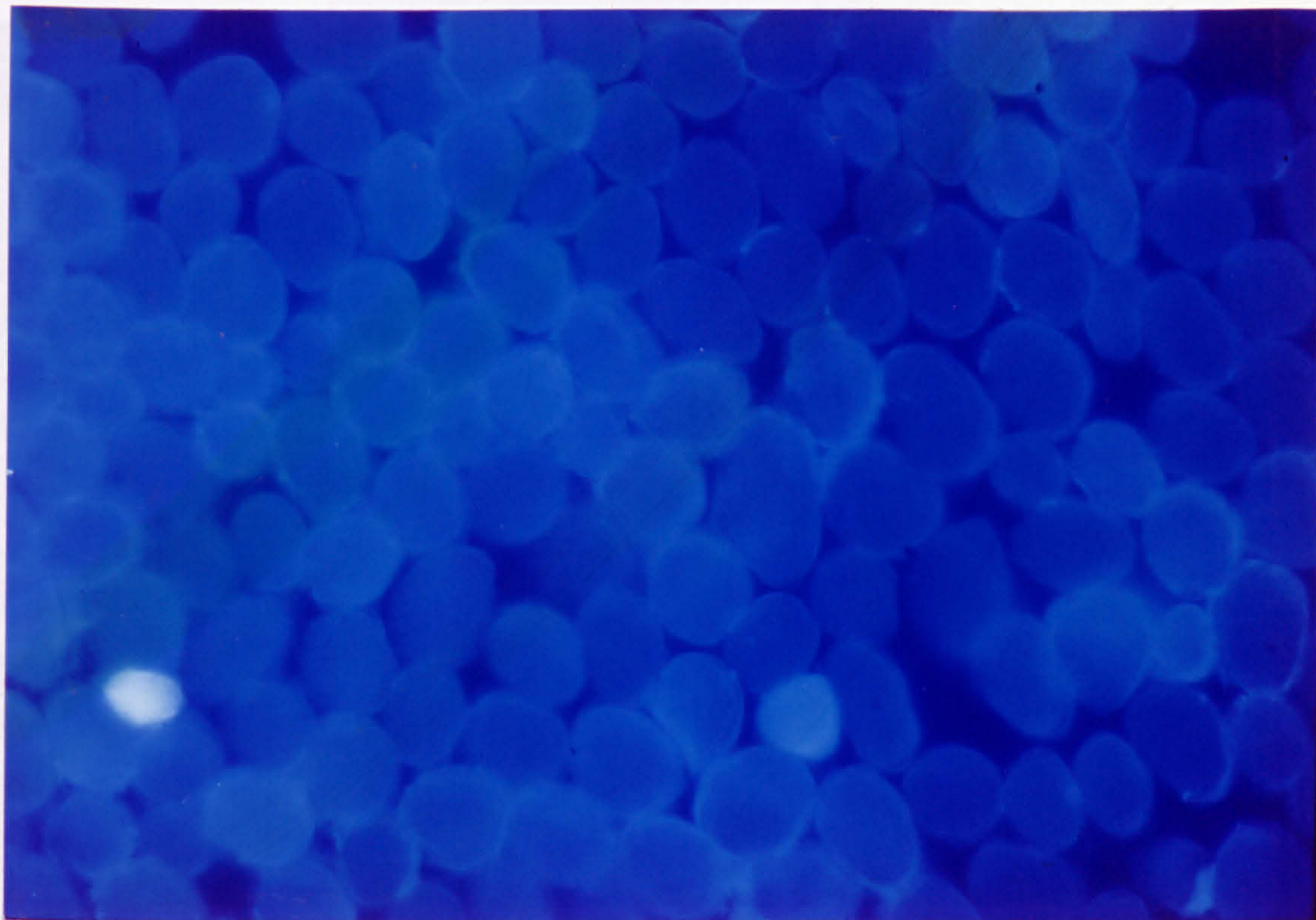


Figure 9.8 Fluorescence micrograph of a cross section of fibres from untreated wool which had been scoured (UV excitation, magnification x450).

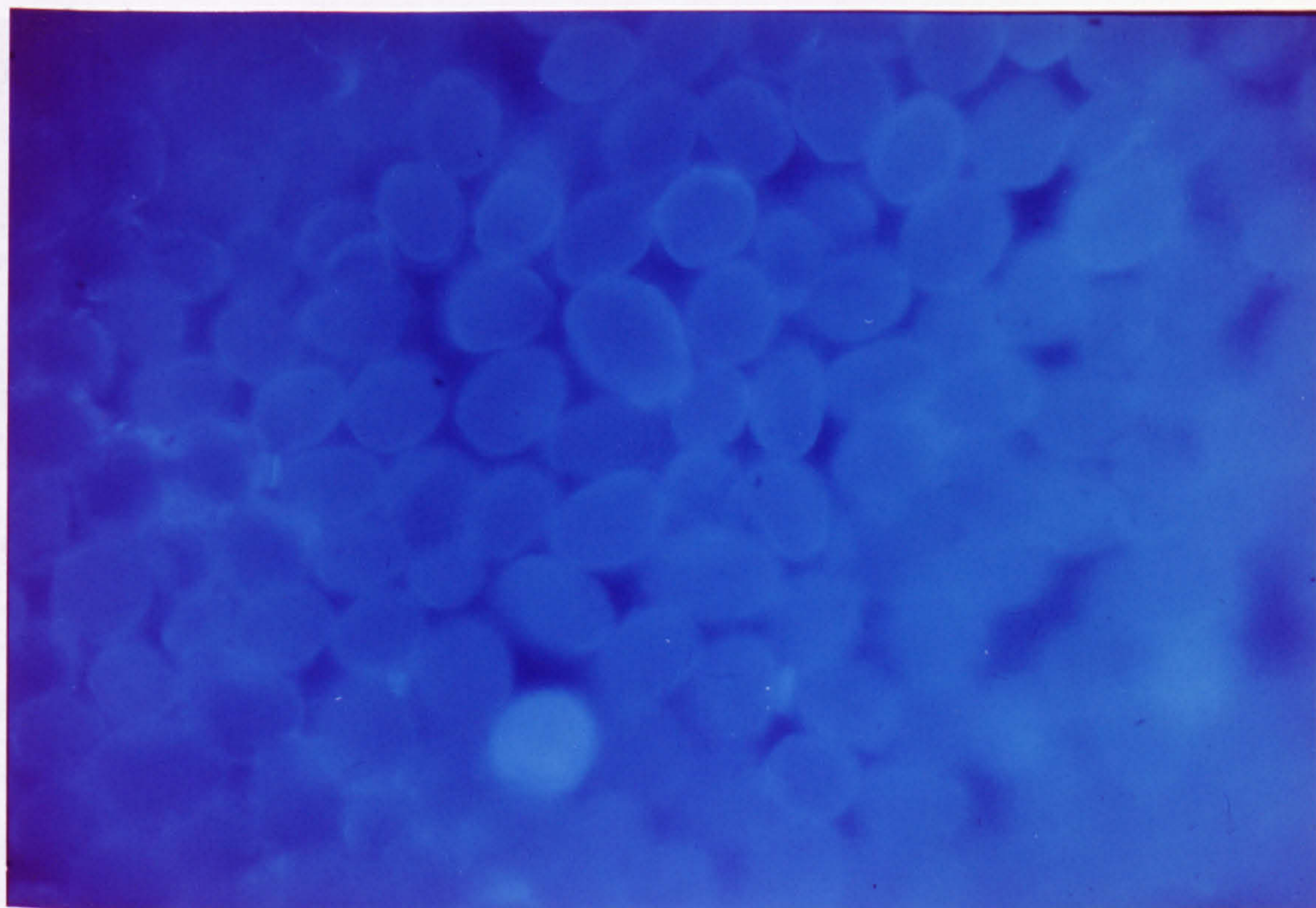


Figure 9.9 Fluorescence micrograph of a cross section of fibres from wool which had been treated with III and left for 39 weeks before being scoured (UV excitation, magnification x450).

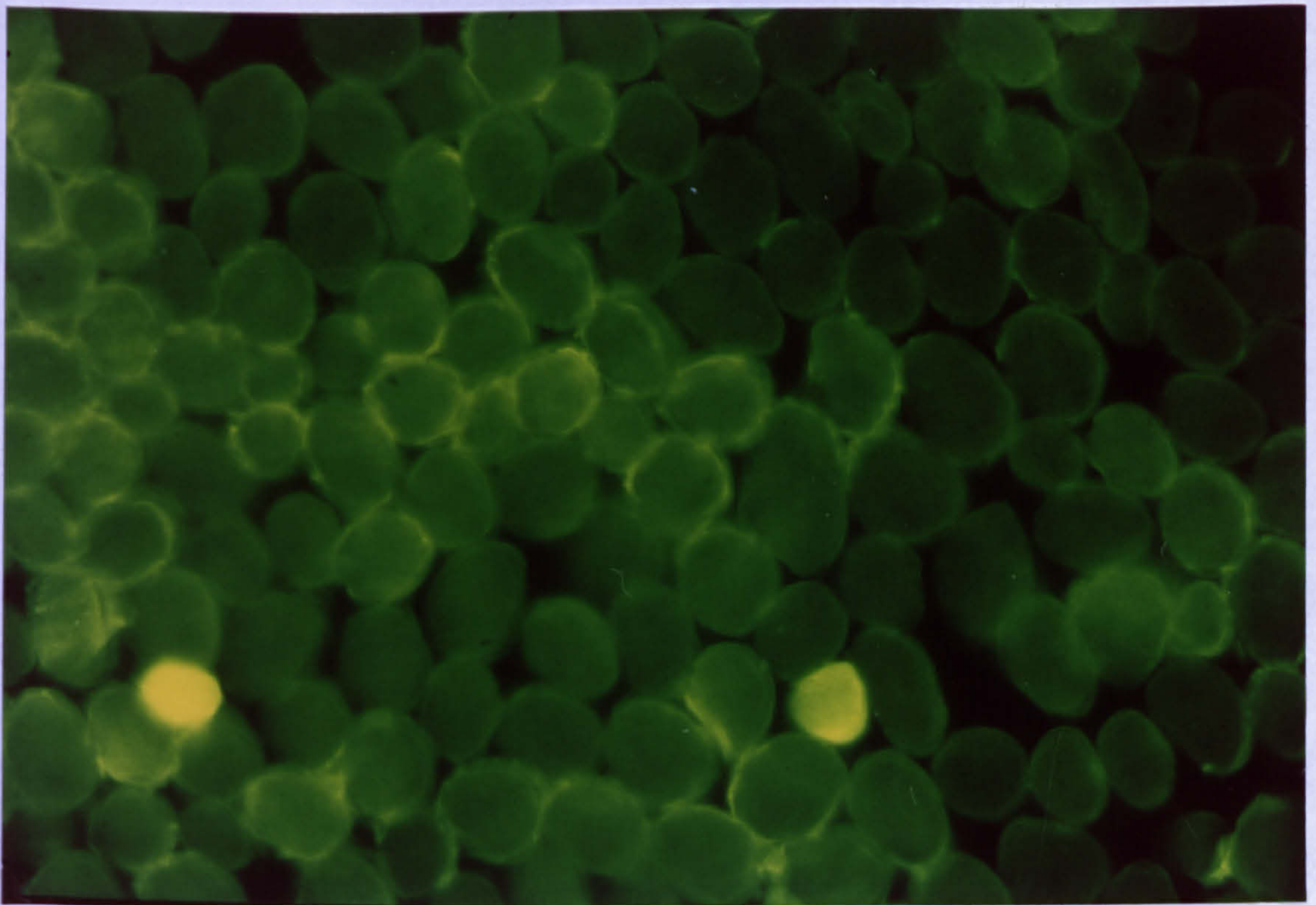


Figure 9.10 Fluorescence micrograph of a cross section of fibres from untreated wool which had been scoured (visible excitation, magnification x450).

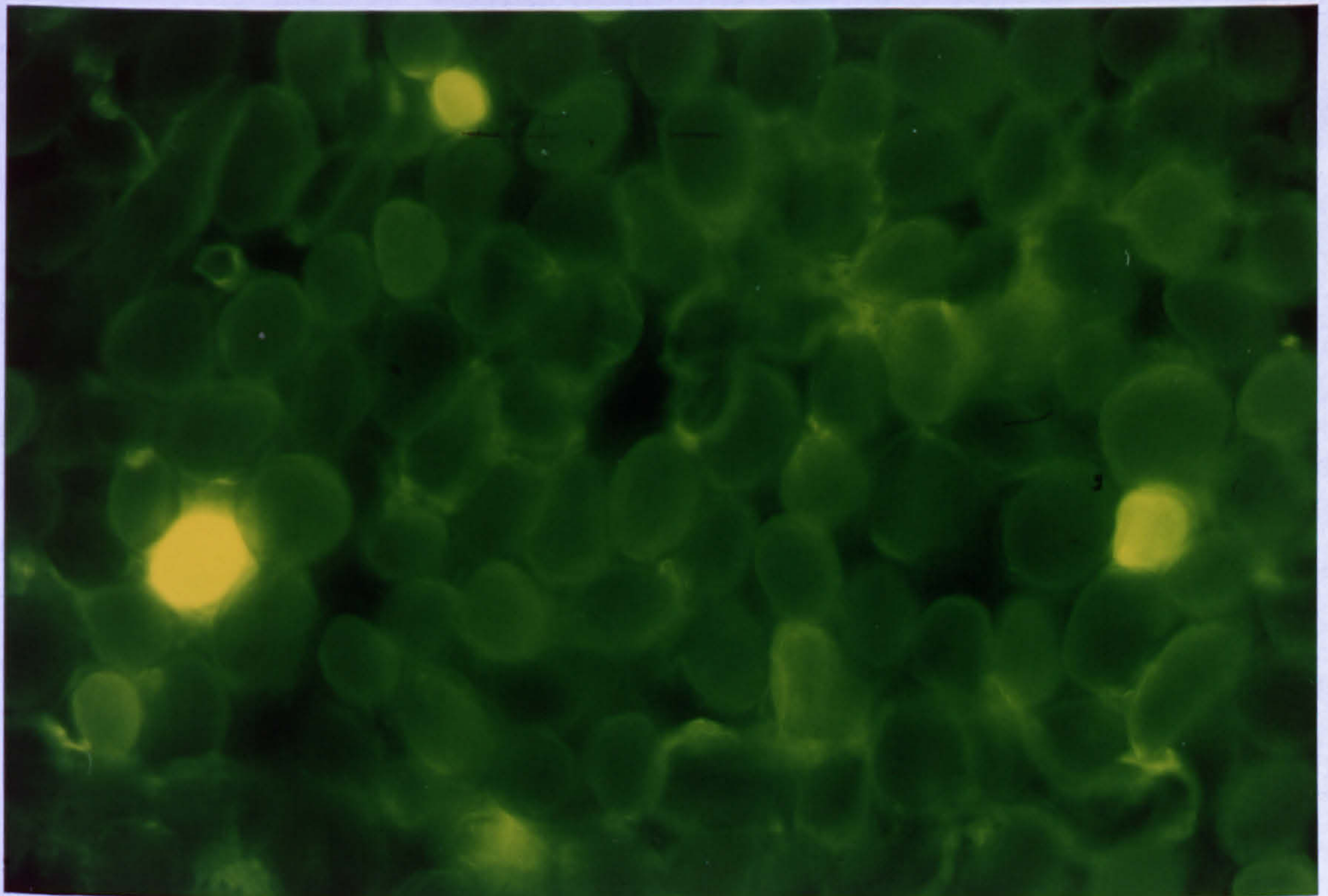


Figure 9.11 Fluorescence micrograph of a cross section of fibres from wool which had been treated with IV and left for 33 weeks before being scoured (visible excitation, magnification x450).

In the present work the scale of the wool cleaned was quite different : 0.54g (III treated), 0.41g (IV treated) and 1.20g (untreated) were each cleaned separately in 1ℓ quantities of water/solution. This is obviously far from normal conditions and may explain the absence of any probe in the fibres - either by the dilution factor (so that it is no longer detectable) or by the absence of a reasonable concentration of suint (obtained by 'continuously' scouring in the same bath), which is known to have scouring properties [30]. An alternative explanation, in the case of IV, is that it may simply have been destroyed by light in the laboratory during the cleaning process. (IV is very light sensitive - see Section 8.3.4.)

These results show that under special conditions no migration into the wool structure was observed. This does not mean that it does not *per se* occur normally in nature. Additional studies could be undertaken, further utilising fluorescent probes. Probes I-III exhibited similar fluorescence (on UV excitation) to that of wool which complicated matters. This can be overcome by using time-resolved microspectrofluorimetry (if the probe has a longer lifetime than the wool) or by using a repeat of the current process but with probes which fluoresce with markedly different wavelength maxima to that of wool e.g. Rhodamine B [21-23]. Rhodamine B has the additional advantage of being more light stable than IV.

These studies cannot be done in a hurry. Sheep are normally sheared annually giving contaminants up to 52 weeks to migrate into the fibre. In the present work the probes had up to 39 weeks (in the case of III) in which to migrate.

9.3.2 Effect of Sensitisers

The possibility of internal sensitisation was investigated by applying chemicals to wool staples under 'swelling' conditions (to ensure uptake). One part of each wool staple was then exposed to UV irradiation (to see the photosensitising effect of the chemical) while the other was left for a while before being cleaned (to see the effect of the chemical on its own (c.f. Section 7.3.3)). The results are presented in Table 9.1.

Treatment	Fluorescence Intensity			
	Before Irradiation ^a		After Irradiation ^a	
	mean ^b	standard deviation	mean ^b	standard deviation
Blank-no solvent	55.3 ^c	11.0	50.5 ^c	13.1
Solvent	42.0 ^c	11.5	90.5	24.5
I + solvent	46.3	13.1	85.1	34.9
II + solvent	46.9	14.6	98.5	25.8
V + solvent	42.9	13.5	90.1	41.5

^a fibres cleaned before measurement except for those marked 'c'

^b arbitrary units, n=15 ^c fibres not cleaned before measurement

Table 9.1 Relative fluorescence intensities of fibres before and after irradiation with UV light for 7 days which had previously been treated with various sensitisers from a 'swelling' solvent (toluene/methanol, 9:1).

From the results of Chapter 6 it would be expected that UV irradiation of untreated wool would cause an increase in fluorescence intensity. The results for the blank in Table 9.1 show that the samples before and after irradiation have approximately the same level of fluorescence. This can be explained by the relatively large standard deviations encountered.

For each of the three sensitisers used (I(1,6-diphenyl-1,3,5-hexatriene), II (parinaric acid) and V (*meso*-tetraphenylporphyrin)) the samples before irradiation have approximately the same level of fluorescence indicating that the chemicals did not have any effect on their own and that the cleaning procedure was successful in removing them.

For each of the three sensitisers used the samples after irradiation have approximately double the level of fluorescence compared to the samples before irradiation. It is also apparent that the three sensitisers have a similar effect to each other. However, treatment with solvent alone had the same effect - an approximate doubling in the level of fluorescence. Consequently, it is clear that

the chemicals are not acting as photosensitisers but that the solvent, toluene/methanol (9:1), is. This is not what was anticipated but it is not unique. It is already known that chlorinated solvent retention promotes photoyellowing [31]. Obviously, the problem of solvent retention is more widespread than was first envisaged.

Recently, after this investigation was carried out, Sideris *et al.* [22] have reported on the photomodification of fibres which had been treated with methylene blue, a powerful photosensitising dye. The methylene blue was deposited into different regions of the fibre depending on the solvent treatment used. Use of anhydrous ethanol/methanol (9:1) restricted dye penetration to the cell membrane complex, while water restricted dye penetration to the orthocortex. After exposure to light (≥ 460 nm) changes were observed in dyeability (a decrease), alkali solubility (an increase) and composition of proteins extracted in urea/dithiothreitol (a decrease). While they made comparisons with undyed and/or unirradiated samples no effect of the solvents on their own was reported. In the light of the results presented here, they may have made a serious oversight.

9.4 CONCLUSION

Under the special conditions used, chosen to mimic natural conditions, no migration of fluorescent probes into the wool structure could be observed. The use of a small laboratory scale scouring process also failed to manifest any signs of migration although such had previously been reported [29]. Additional studies further utilising fluorescent probes could be undertaken. However, if the answer is negative in nature then no evidence will ever be found.

Application of sensitisers from a 'swelling' solvent, toluene/methanol (9:1), failed to lead to the chemicals acting as photosensitisers. However, the photosensitising property of the solvent was revealed. This indicates the importance of care in the use of solvents and the necessity of carrying out appropriate blank experiments for comparison.

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EPILOGUE

'Blessed is the man who finds wisdom,
the man who gains understanding,
for she is more profitable than silver,
and yields better returns than gold'.

Proverbs 3.13,14

'Of making many books there is no end, and much study wearies the body.
Now all has been heard;
here is the conclusion of the matter:
Fear God and keep his commandments,
for this is the whole duty of man.
For God will bring every deed into judgement,
including every hidden thing, whether it is good or evil'.

Ecclesiastes 12. 12-14